EVALUATION OF BRAINSTEM AUDITORY EVOKED POTENTIAL AND SERUM INTERLEUKIN-1BETA LEVELS IN PATIENTS WITH GENERALIZED TONIC CLONIC SEIZURES

Dissertation submitted to

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

In partial fulfillment of the regulations

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Branch V

INSTITUTE OF PHYSIOLOGY & EXPERIMENTAL MEDICINE

MADRAS MEDICAL COLLEGE AND GOVERNMENT

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April – 2015

CERTIFICATE

This is to certify that the dissertation entitled "Evaluation of Brainstem auditory evoked potential and serum Interleukin-1beta levels in patients with generalized tonic clonic seizures" by the candidate Dr. G. Savitha for M.D Physiology is a bonafide record of the research done by her during the period of study (2012-2015) in the Institute of Physiology and Experimental medicine, Madras Medical College, Chennai-600003.

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ABSTRACT

EVALUATION OF BRAINSTEM AUDITORY EVOKED POTENTIAL AND SERUM INTERLEUKIN-1BETA LEVELS IN PATIENTS WITH GENERALIZED TONIC CLONIC SEIZURES

Degree for which submitted	:	Doctor of Medicine(MD) in Physiology
Supervisor and guide	:	Prof.Dr.K.Padma Director and Head of the Department
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Year	:	2014

BACKGROUND

Epilepsy is a neurological disorder characterized by abnormal changes in the brain's electrical potentials. Brainstem Auditory Evoked Potential (BAEP) is an important clinical tool in studying the electrophysiological phenomena of neural excitation, conduction and transmission across the auditory pathway. It has been widely used for the early detection of neural conduction irregularities in auditory pathway. Using BAEP, we can easily detect the early changes occurring in the auditory pathway in Generalized

Tonic Clonic Seizure(GTCS) patients even before the clinical manifestation of hearing impairment occurs so that proper measures to intervene the disease process at the earliest possible is achieved to provide a better quality of life for GTCS patients.

AIM OF THE STUDY

- To determine the functional integrity of auditory pathway in patients with GTCS by recording brainstem auditory evoked potential.
- To assess serum Interleukin -1 beta levels in these patients.
- To find the correlation between serum Interleukin-1 beta level and Brainstem auditory evoked potential in patients with GTCS.

MATERIALS AND METHODS

30 patients with GTCS of both sexes in the age group 20-40 years without any clinical evidence of hearing impairment were included in the study. Controls were age, sex and BMI matched healthy population. Both the controls and GTCS patients were subjected to BAEP and serum interleukin-1beta levels were also measured. The data were analyzed by Student ‘t’ test.

RESULT

GTCS patients showed significant differences in wave III, V absolute latency and I-III, I-V IPL of BAEP. The interleukin-1β levels were significantly prolonged in GTCS patients as compared to control population suggesting the contribution of this cytokine in epileptogenesis.

CONCLUSION

There was significant prolongation of central conduction time in GTCS patients even though there was no clinical evidence of hearing impairment assessed by pure tone audiogram prior to the study. Hence BAEP can be utilized as an objective electrophysiological tool to evaluate the functional integrity of auditory pathway from the external ear to lower brainstem.

KEY WORDS

Generalized tonic clonic seizures, Brainstem auditory evoked potential, Absolute latency, Interpeak latency.

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GLOSSARY OF ABBREVIATIONS

ABR	Auditory Brainstem Response
AVCN	Anterior Ventral Cochlear Nucleus
AP	Action potential
BERA	Brainstem Evoked Response Audiometry
BAEP	Brainstem Auditory Evoked Potential
CNS	Central Nervous system
CPS	Complex Partial Seizure
CSF	Cerebrospinal fluid
DCN	Dorsal Cochlear Nucleus
dB	decibel
EPSP	Excitatory Post Synaptic potential
GABA	Gamma Amino Butyric Acid
GTCS	Generalized Tonic Clonic seizure
ILAE	International League Against Epilepsy
IL-1β	Interleukin -1 beta
IPL	Inter Peak Latency
IPSP	Inhibitory Post Synaptic Potential
IBE	International Bureau of Epilepsy
MGB	Medial Geniculate Body
ms	milliseconds
PVCN	Posterior Ventral Cochlear Nucleus
pg	picogram
NTS	Nucleus Tractus Solitarius
RMP	Resting Membrane Potential
SPS	Simple Partial Seizure
SUDEP	Sudden Death
WHO	World Health Organisation

1.INTRODUCTION

1. INTRODUCTION

A seizure (derived from Latin word Sacire meaning to take possession of) is a paroxysmal event which occurs due to abnormal excessive or synchronous neuronal activity in a discrete or generalized portion of brain.

Seizures can be provoked by acute brain insult or systemic diseases like stroke or metabolic causes but if it occurs in the absence of an acute provoked event, it is termed as unprovoked seizure[1]. Epilepsy is defined as the tendency to have at least two episodes of unprovoked seizures separated by a minimum period of 24 hours.

In 2005, International League Against Epilepsy (ILAE) and International Bureau of Epilepsy (IBE) proposed the definition of epilepsy as a disorder of brain by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences of this condition.

Efforts to classify epileptic seizures date back to the earliest of medical literature. In 1964, the Commission on classification and terminology of ILAE proposed the first official classification of epileptic seizures which was revised in 1981[2,3].

ILAE classification of epileptic seizures

I. Partial (focal, local) seizures

A. Simple partial seizures (consciousness not impaired)

-with motor symptoms

-with somatosensory or special sensory symptoms

-with autonomic symptoms

-with psychic symptoms

B. Complex partial seizures (with impairment of consciousness)

-with simple partial onset followed by impairment of consciousness

-with impairment of consciousness at onset

C. Partial seizures evolving to secondarily generalized seizures

-SPS evolving to generalized seizures

-CPS evolving to generalized seizures

-SPS evolving to CPS evolving to generalized seizures

II. Generalized seizures (convulsive or non -convulsive)

-Absence seizures

1. Typical absence seizures

2. Atypical absence seizures

-Myoclonic seizures

-Clonic seizures

-Tonic seizures

-Tonic clonic seizures

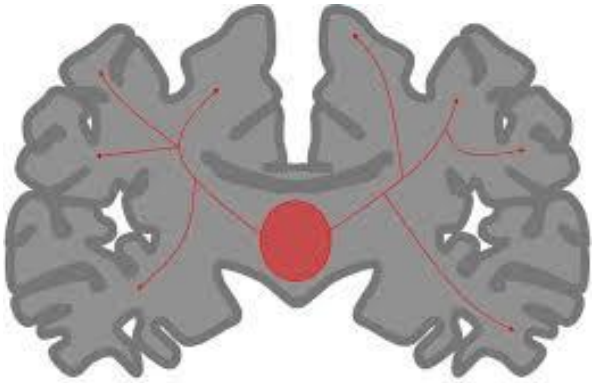
-Atonic seizures

III. Unclassified epileptic seizures

The ILAE described generalized seizures as “In seizures that are generalized at onset, the abnormal activity probably originates in the central mechanisms controlling cortical activation and then it spreads rapidly”. So generalized seizures are rightly defined as originating at some point within and rapidly engaging, bilaterally distributed networks which does not necessarily include the entire cortex. They begin with simultaneous and almost equal involvement of both cerebral hemispheres from the onset and

involve the deeper thalamic, subcortical and brainstem structures in a feedback loop to the cortices.

FIGURE 1: Cross section of brain showing primary generalized seizure



Generalized tonic clonic seizures are the most commonly encountered seizures in both children and adults, the classic picture which the public generically perceives as epilepsy. The glossary of descriptive terminology for ictal semiology provided by ILAE taskforce describes a tonic clonic seizure as "a sequence consisting of a tonic followed by a clonic phase .Variants such as clonic-tonic –clonic may be seen"[102].

A GTCS may be of generalized onset (primarily GTCS) or it may begin focally, followed by secondary generalization (secondarily generalized). The clinical manifestations observed in these seizures are initiated by abnormal electrical discharges within the brain and depend on the part of brain involved in epileptic neuronal discharge and the intensity of discharge. They are sudden, transient and usually brief which include motor,

psychic, autonomic and sensory phenomena with or without alteration in consciousness and awareness.

Brainstem auditory evoked potentials are electrophysiological recordings of responses from within the auditory system that are activated by sounds. They are recorded from the ear and vertex in response to a brief auditory stimulation to assess the conduction through auditory pathways up to midbrain. The human Brainstem auditory evoked potentials (BAEP) consist of far field evoked potentials from the auditory nervous system. The BAEP is the averaged surface recorded activity from multiple source neural generators in the peripheral and lower central auditory nervous system and represents the synchronous discharge activity of onset –sensitive single units from first through sixth order neurons. The evoked transient responses can be recorded up to 500 ms from time of onset of the sound stimulus. The evoked potentials of the first 10 ms i.e.) short latency response (SLR) is popularly known as Brainstem auditory evoked potentials (BAEP).

BAEP comprises of 5 or more waves within 10 ms of stimulus and 3 interpeak latencies. Each individual wave and interpeak latencies provide information about an area of auditory pathway starting with cochlear nerve to the level of inferior colliculi. These were first described by Jewett and Williston in 1971.

Epilepsy is a neurological disorder characterized by abnormal changes in the brain's electrical potentials. The dysfunction occurring at the cellular level leads to excessive neuronal excitability and AEPs are expected to be altered by such cellular dysfunction. Hence BAEPs have emerged as an important clinical tool in studying the electrophysiological phenomena of neural excitation, conduction and transmission across the auditory pathway (Tandon OP et al 1990)[4].

Cytokines are soluble potent glycoproteins secreted by the glial cells of CNS and they function as immune system mediators. They mediate cell to cell sigalling by binding to high affinity surface receptors and serve as a biomarker for earlier detection of brain damage to prevent further neurological complications. Abnormalities in the expression of cytokines and immune cells is noted in epilepsy patients and in various animal models (Jobe PC et al 1991)[5] and hence the immune system and its associated inflammatory reactions appear to play a major role in epileptogenesis and aggravate brain damage (Holmes et al 2002)[6]. Epilepsy per se is capable of producing elevated levels of cytokines. Such elevated levels as during inflammation of brain or periphery decreases seizure threshold and predisposes to epilepsy.

Interleukin-1beta (IL-1β) is one such pro inflammatory cytokine released from glial cells during seizures. Many CNS diseases such as seizure and cerebral ischemia are associated with BBB leakage which leads to

extravasation of CNS-foreign proteins like albumin and simultaneously the excitotoxic damage produced by such diseases causes increased microglial IL-1β expression. This leads to reduced seizure threshold and epilepsy which depends on the amount of neuronal IL-1RI and IL-1RII.

The chronic expression of IL-1β during epileptogenesis contributing to neuronal injury suggests that IL-1 β activated pathways play a vital role in the genesis of spontaneous seizures, thus raising the possibility of using IL-1 antagonist as a novel drug for seizure inhibition in clinical practice (Randle et al 2001)[103]. IL-1 production and activity are regulated by many factors like caspase-1, IL-1RI, IL-1RII, IL-Ira which implies there are many ways to interfere with IL-1β activity, of which the anticonvulsive effects can be explored. IL-1β also influence many central neurotransmitters including GABA, 5-hydroxy tryptamine, noradrenaline and acetyl choline as well as expression of a number of neuropeptides in several brain regions contributing to changes in auditory evoked potentials of GTCS patients.

Hence considering the above factors, the present study is undertaken to assess the functional integrity of auditory pathway using Brainstem evoked response audiometry in patients with generalized tonic clonic seizures. We also compare serum Interleukin-1β levels in patients with GTCS and normal controls and then correlate their levels with BAEPs in GTCS patients.

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Seizure is one of the most dramatic example of the collective electrical behaviour of the mammalian brain It is generally a chronic problem with significant impact on personal, social and economic aspects, often affecting the ability to hold jobs and drive. Generalised tonic clonic seizures are the best recognised form of seizures often presenting with loss of consciousness and a generalised tonic contraction evolving gradually into clonic activity.

BAEP is an objective neurophysiological test used to evaluate the neural activity from the external ear to lower brainstem. This non-invasive tool can be utilized in GTCS patients for early detection of neural conduction irregularities in the auditory pathway. A few studies in this field of research have shown an increase in the latency of BAEP waves and so with this background , the present study has been taken up to assess the integrity of auditory pathway in GTCS patients by recording brainstem auditory evoked potentials.

As several researches have high-lighted the intrinsic role of interleukin-1β in the process of epileptogenesis, the present study is aimed at measuring serum Interlukin-1β levels in GTCS patients so that the antagonists of this cytokine can be used in clinical practice for seizure inhibition in near future.

Epilepsy had been one of the earliest and commonly recognised neurological disorders (Temkin O et al 1994)[7]. The earliest references to the disorder dates back to second millennium BC where Mesopotamian writings described it as antasabbu literally meaning the falling disease. Indian Ayurvedic writings roughly belonging to the same period also contain elaborate clinical description of epilepsy as ashepak or apasmara. In 400 BC, epilepsy was called sacred disease because people believed that people suffering from seizures were possessed by evil spirits or gods and should be treated by the invocation of religious, occult and magical powers.

Hippocrates, the Father of Medicine made the notable conceptual contribution saying epilepsy is an illness as any other disease and no more considered divine or spiritual. He proposed that brain is the organ where the site of seizure onset is located and also revealed the existence of genetic basis in epileptic patients.

Galen, a Greek physician introduced the term aura to describe the symptoms that preceded the onset of epilepsy.

Tissot recognised two types of ictal events, GTCS which he called grands acces and absence seizures as petits acces.

In 1873, David Ferrier the Scottish neurologist clearly demonstrated in

monkeys that motor cortex and not medulla initiated the convulsive motor activity.

John Hughlings Jackson (1835-1911), the father of epilepsy confirmed the relationship that existed between the structure and physiology of the nervous system to elucidate the pathophysiology associated with seizures. He also supported the findings of David Ferrier regarding the site of seizure onset and implicated that the ictal behaviour correlated well with the region of functional anatomy. The potential therapeutic importance of his intellectual conclusion was that the surgical treatment may be effective as an underlying pathology or structural lesion was presumed to be associated with the site of epileptogenesis.

Victor Horseley performed the first surgery for epilepsy in 1886 by resecting a traumatic cortical scar in a patient with focal motor seizure rendering him seizure free.

Hans Berger's invention of electroencephalogram in 1929 made a historical revolutionary impact on the diagnosis of epilepsy.

2.1.1. Epidemiology

The incidence of epilepsy is 0.3-0.5% in the world population which is age dependent. Along with various studies, Hirtz D et al also have shown that higher rates occur in infants younger than one year and a second peak is found in people older than 60 years presenting with bimodal distribution[8]. Seizures are so common to occur in 10%of the population at some point in their lifetime (Berg et al 1991)[9]. Sex specific incidence rates are not usually significant, although the incidence rates are almost always higher for males as compared to females. The prevalence is estimated to be 5-10 persons /1000 which is higher in developing countries. Sridharan et al estimated the prevalence in Indian population to be 1% which is higher in rural than urban population [10].

Perhaps more important than incidence and prevalence in understanding the impact of epilepsy as a worldwide health problem is the global burden of the disease which is measured by Disability Adjusted Life Years(DALYs) and number of life years lost due to disability or death(YLL). According to WHO, epilepsy was estimated to account 0.5% of the global burden of disease with 7.3 million DALYs and 1.32 million YLL[11].

2.1.2 Etiology

1. In 60-70% of patients with seizures, no specific cause is identified which is commonly referred as idiopathic.

2. Genetic- risk is increased 2-3 times in individuals having first degree relatives with epilepsy.

3. Infants and children- congenital malformations, perinatal injuries or hypoxia, developmental neurological disorders, metabolic defects, injuries and infections.

4. Young adults- head trauma, brain tumours, infections and arteriovenous malformations.

5. Elderly – cerebrovascular disease, CNS degenerative diseases, brain tumours.

6. Drugs- antihistamines, narcotic analgesics and iodinated contrast agents like metrizamide (Messing RO et al 1984) [12].

2.1.3. Genetics and Epilepsy[13]

Most of the seizures are believed to be complex traits resulting from interactions between non genetic and genetic factors, the latter thought to provide minor contributions from multiple genes (oligogenic or polygenic) and thus the patterns of inheritance in majority of seizures are complex and subtle.

Many generalised seizures in humans have revealed a genetic basis and these almost become apparent before the age of 35 years. Recent studies suggest that the mutations affecting the ion channel function and chromosomal microdeletion may be the cause of epilepsy in a subset of patients.

Tan NC et al[14] have quoted many association studies that have been undertaken to examine the influence of common genetic variation on disease susceptibility in epilepsy, but unfortunately no genetic variants have been proven to underlie any common epilepsy. Studies in twins have demonstrated concordance rates as high as 70% in monozygotic and 10% in dizygotic pairs implying that factors other than genetics play a role in the occurrence of seizures (Treiman et al 1993)[15].

Triggering factors for seizures include

-Sleep deprivation

-Physical and mental exhaustion

-Flickering lights including television and computer screens

-Alcohol (particularly withdrawal)

-Intercurrent infections or metabolic disturbances

-Missed doses of antiepileptic drugs in patients undergoing treatment

2.1.4. Comorbidities in Epilepsy

Individuals with epilepsy are at risk for increased morbidity and mortality as compared to general population. Patients with drug resistant epilepsy and longer duration of the disorder contribute significantly to higher morbidity in various surveys due to the result of stress and its adverse effects on various systems. In patients with newly diagnosed epilepsy, 18% of them present with additional dementia, 6% motor disabilities and 6% with severe psychiatric disturbances. So about 1 in 15 of patients with seizures depend on others for daily living due to associated handicaps. Poor epilepsy control and seizure themselves can lead to significant cognitive and personality changes as well as chronic depression. It results in social stigmatisation resulting in isolation which further creates problem in education, employment, personal relationships and family life.

Patients with epilepsy have 2-3 times greater risk of mortality than expected in a matched population without epilepsy. SUDEP accounts for about 1.7% deaths in epilepsy which when witnessed is most often associated with GTCS near the time of death.

2.1.5. Pathophysiology of Seizures

The electro physiologic and molecular mechanisms that underlie the pathophysiology of seizures is poorly understood. Animal models using

maximal electroshock or chemoconvulsants such as pentylenetetrazol have been utilised to probe into the pathophysiology of focal and generalised seizures.

Seizures are linked at the lowest level to membrane potentials, ionic fluxes and generation of action potentials. The RMP of the neuronal membrane is approximately about -70mV. In neurons the action potential is generated due to changes in the permeability of sodium, chloride, calcium and potassium ions which enter and exit the neurons by voltage dependent channels. At threshold voltage there is Na^+ influx due to high permeability of these ions, the membrane potential becomes dramatically positive (+60 mV) which generates the action potential. After 1ms Na^+ channels are inactivated followed by K^+ efflux and the coincident Cl^- influx result in membrane hyperpolarisation and the termination of electrical activity at that point along the cell membrane (Hille B et al 1984)[16].

As the action potential reaches the axon terminal, voltage dependent Calcium channels open, permitting Calcium influx into presynaptic terminals which results in neurotransmitter release. Hence any changes or abnormalities at any point along this electric cascade will have a significant impact on the excitability and epileptogenecity of the individual neuron (Mc Namara JO et al 1994) [17].

The effects of neuronal activity are mediated through synaptic connections existing between neocortical, thalamocortical and corticothalamic projections. The impulses finally culminate in either excitatory (EPSP) or inhibitory postsynaptic potentials (IPSP). Excitatory inputs are transmitted by neurotransmitters like glutamate and aspartate whereas inhibitory inputs are mediated by gamma aminobutyric acid (GABA).

Seizure propagation is likely to occur in cells with intrinsic bursting if the balance between excitatory and inhibitory inputs is altered. Neurons begin to fire synchronously leading to a seizure in the absence of appropriate inhibitory regulation (loss of IPSPs).

Goldensohn et al[18] proposed a hypothesis that the neurons within the epileptic focus of cortex undergo paroxysmal synchronous depolarisation termed as paroxysmal depolarizing shift(PDS) resulting in an abnormal burst of action potentials which continue in synchronous volleys without appropriate inhibition.

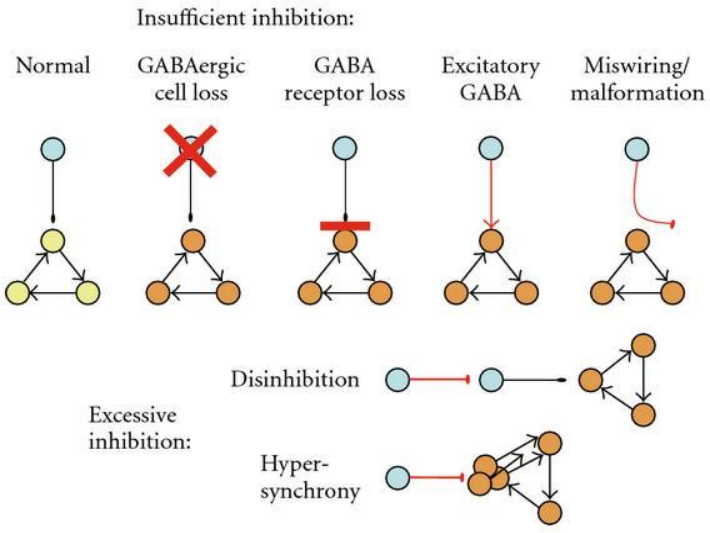
2.1.6. Neurotransmitters

GABA is found to be a critical inhibitory modulator of neuronal activity in the brain (Gale K et al 1992)[19]. It generates IPSPs mediating synaptic inhibition which counterbalances the excitatory inputs from other

regions of brain. Three distinct types of GABA receptors are identified. GABA-A, B, C. GABA-A receptor, a pentameric complex is organised to form a chloride channel through which Cl^- ions can enter into the postsynaptic region. When GABA binds with GABA-A receptor, there is influx of Cl^- ions resulting in generation of IPSP. Wallace et al[20] found that mutations of GABRG2 gene encoding for GABA-A subunit leads to generalised and febrile seizures.

Lothman E et al[21] suggest that disruption of GABAergic function may be central to the molecular pathophysiology of seizures. Hence interruption of integrated GABA mediated inhibition in cortex will result in a lowered threshold for ictal discharges. Various animal models have supported this mechanism which shows that GABA agonists prevent seizures while antagonists may provoke seizures.

FIGURE 2: GABA in epileptogenesis



Glutamate and aspartate are excitatory neurotransmitters which act by binding to EAA receptors resulting in enhanced intracellular Calcium levels. This causes activation of proteases and lipases and osmotic swelling of neurons resulting in cell death.

Meldrum B et al proved in his study that glutamate and aspartate contribute to the initiation, spread and maintenance of epileptic activity in cortex[22].

During MJ et al studied in epilepsy patients to confirm increased levels of EAA compounds such as aspartate using micro dialysis catheters immediately prior to the onset of seizure activity[23].

2.1.7. Effects of Seizures on Brain

Ischemia /infarction, trauma and seizure are three major forms of acute brain injury which leads to significant neuronal loss resulting in neuronal dysfunction. There may be some common cellular mechanisms involved in these pathologies, but the induction of cell death following seizure activity is probably less well understood than other forms of brain injury (Liou AK et al 2003)[24].

Delorenzo RJ et al observed that following a prolonged seizure, there is excessive release of excitatory neurotransmitter, which activates N methyl D

aspartate(NMDA) receptors and voltage activated Calcium channels that results in cellular influx of Calcium ions[25]. An increase in free calcium tends to produce mitochondrial dysfunction with activation of various enzymes like lipases, endonucleases, proteases etc. In addition, free radicals are generated contributing to mitochondrial dysfunction[26] and ultrastructural changes may be observed after 30-60 min of seizures (Meldrum BS et al 2002)[27]. This results in reversible or irreversible neuronal damage and CSF markers of neuronal injury like neuron specific enolase increase following seizure activity (Pitkanen S et al 2002)[28].

Prolonged seizures may ultimately result in neuronal death by either necrotic or apoptotic pathways. Based on classic morphological definitions, cell necrosis appear to be the dominant mechanism, the exception being granule cell death in the dentate gyrus including recently generated neurons in this region which more readily show apoptosis (Ekdhal CT et al 2003)[29]. However animal models suggested varying ratios of apoptic vs necrotic cell death suggesting heterogeneity in the cellular effects of seizures. This state of flux is found to be dependent on energy state of the cell and on the time course and severity of the neurotoxic insult, cell necrosis being observed with severe excitototoxic insults and apoptosis with milder insults to more resilient neurons which allows the induction of more delayed energy dependent cell pathways (Kondratyev A et al 2004)[30].

Thus epileptic seizures can cause severe and long lasting events on the brain architecture including neuronal cell death, accompanied neurogenesis, reactive gliosis and mossy fibre sprouting.

2.1.8. Generalised Tonic Clonic Seizures

Gestaut and Broughton gave an elaborate description of the semiology and pathophysiology of GTCS[32]. By stressing the stereotypical nature of GTCS, they divided it into 4 distinct phases

1. Preictal manifestations

2.Ictal manifestations (with loss of consciousness)

   a. Tonic phase (includes intermediate vibratory phase)

   b. Clonic phase

   c. (concurrent) Autonomic changes

3. Immediate postictal features

4. Late postictal features

According to Gestaut and Broughton, preictal manifestations are brief with bilateral myoclonic contractions which immediately precedes the onset of tonic phase. But Fisch BJ et al[33] defines it as prodromal phase presenting

with nonspecific symptoms such as headache, irritability, lethargy, mood changes and sleep disturbances. These symptoms actually do not represent epileptic aura but reflect the physiological changes that reduce the seizure threshold. If aura occurs before a seizure, it indicates that the tonic clonic seizure is secondarily generalised.

GTCS synonymous with the previous term grandmal epilepsy or convulsions is the classical and best recognised form of seizures which typifies epilepsy in public imagination. These seizures do not have an aura but preceded by a prodrome that occurs for minutes to a few hours before a seizure which includes inconsistent nonspecific premonitory symptoms like ill-defined anxiety, instability, reduced concentration, headache or vague uncomfortable feelings.

The seizure onset is abrupt often presenting with loss of consciousness followed by the tonic phase. In this phase the patient tends to fall if he is standing with bilateral tonic extension of trunk and extremities followed by synchronous muscle jerks. This phase can have asymmetrical movement which often varies from seizure to seizure and one such commonly observed asymmetry is versive head turning which is not an evidence of focal onset. It also includes upward deviation with partially opened eyes and mouth is also opened.

Involvement of respiratory muscles produces epileptic cry which is characterised by forced expiration that produces a loud guttural localisation. Cyanosis may occur in this phase in association with apnoea. Autonomic signs are present during this phase which includes tachycardia, hypertension, cyanosis, salivation, sweating and incontinence of urine or stools.

Benarroch EE et al was able to elucidate that increase in heart rate and blood pressure was either mediated directly through the ictal activation of structures of the central autonomic network which includes insular cortex, amygdala, hypothalamus, periaqueductal grey matter, para brachial complex, NTS and ventrolateral medulla or reflecting the high metabolic demand of the seizure.

Plum F et al[34] reported that the rise in blood pressure evoked by seizure usually causes a significant increase in cerebral blood flow to meet the increased metabolic demands of brain.

Bateman LM etal observed that when diaphragm and thoracoabdominal muscles are involved during the tonic phase, it results in insufficient air exchange, which in turn may lead to alveolar hypoventilation causing decrease in blood O2 saturation and cyanosis.

This stage lasts on an average of 10-30 sec and gradually evolves into clonic activity. The transition can be initially of high frequency and low amplitude motion often referred as the vibratory phase.

As the cortical discharges diminish with frequency, the clonic phase ensues where the limbs produce repetitive myoclonic jerks for a variable time. With seizure progression, the frequency of clonic jerks reduces and the amplitude which rises initially also decreases just before the seizure stops. The final phase lasts for 2-30 min and is characterised by flaccidity of muscles and diminished tendon jerks.

Confusion and unresponsiveness is invariable in the postictal period. Respiration is loud and stertorous in nature. In this phase the patient feels dazed with a severe headache and extremely unwell, he often lapses into a deep sleep. On awakening within minutes or hours later, some residual symptoms may persist which include headache, dysthymia, lethargy and generalised muscle soreness. GTCS rarely last greater than 2 minutes and the postictal state is found to be correlated with severity and duration of the seizure episode.

2.1.9. Diagnosis of Epilepsy

In assessment of a patient with epilepsy, the history takes primacy ideally in conjunction with an eye witness account. In a vast majority of cases the diagnosis of epilepsy is clinical but a syndromic diagnosis is also possible on the basis of clinical signs and symptoms.

EEG optimally undertaken with video recording assists in

confirming the diagnosis and type of epilepsy (focal vs generalised) and also in localising the area of seizure onset. EEG must be performed, it is more likely to be abnormal within first 2 days after a seizure[31]. Ictal EEG findings at onset include high amplitude anteriorly dominant generalised spike wave discharges, diffuse fast frequencies that evolve to generalised spike wave discharges or polyspike wave discharges.

Once the seizure is clinically manifest, the muscle activity prevents the determination of EEG changes. Post ictally the EEG often shows diffuse slowing ie) slow spike wave discharges. Inter ictal EEG shows either a normal background or runs of occipital delta activity and may show either fragmented diffuse spike wave or polyspike wave discharges or frank generalised spike wave discharges.

2.2. Evoked Potentials

An evoked potential is an electrical manifestation of the brain's reception of and response to an evoked stimulus. Evoked potentials have been studied in patients with neurological diseases since early 1950s but it was only in the early 1970s that evoked potentials began to have definite clinical utility. These tests provide sensitive and quantitative extensions of the clinical neurologic examination. Brainstem auditory evoked potential has earned a strong clinical reputation as a tool to evaluate the integrity of

auditory pathway from the external ear to lower brainstem and has been extensively used in patients with seizures.

2.2.1. BAEP and epilepsy

Epilepsy is a neurological disorder which is characterized by abnormal changes in the brain's electrical potentials. The excessive neuronal excitability produced as a result of biophysical and biochemical cellular dysfunction alters the BAEP which is noted in various studies.

- Cranfor JL et al[128] has reported that central auditory impairment is present in bi-hemispheric seizure disorders. The patients have shown improvement in the measures of central auditory function following successful surgery to control epilepsy, but the results are prone to variability.

- Zhao JY et al analysed a Chinese family and proved that hearing loss associated with epilepsy is due to a mitochondrial mutation. He evaluated in clinical and genetic aspects along with sequential analysis of mitochondrial genome in a three generation Chinese family and identified that 7472 del C is likely to be a novel mitochondrial mutation associated with hearing loss in epilepsy patients.

- Rodin et al[97] studied 80 epileptic patients and observed significantly

prolonged I-III and I-V IPLs and longer standard deviations as compared to the normal controls. He noted that the number of different seizure types in an epileptic patient was significantly related to the changes in latency but type and duration of seizure do not show any such correlation.

- Salah Soliman et al[98] recorded auditory brain stem response and middle latency response in 49 epileptic patients. He found that a statistically significant number of epilepsy patients showed elevated ABR (30.1%) and MLR (40.7%) in spite of these patients having normal hearing sensitivity which was assessed by pure tone audiograms. He observed that threshold elevation was more frequent in patients with grandmal epilepsy when compared with temporal epilepsy patients reflecting poor response in the former group. Furthermore they also noted that chronicity of illness was significantly related to the elevated ABR and MLR thresholds in patients with grandmal epilepsy in contrast to temporal lobe epilepsy patients.

- Masayuki Ohishi et al[125] selected 114 epileptic patients –both males and females and recorded ABR in these patients. He observed that the female epileptic group showed longer I-V IPL than that of the controls.

- Chayasirisobhon S, Rodin E et al studied the functions of brainstem in 81 epileptic patients using BAEPs. He acknowledged that the epileptic patients had significantly longer latencies for all the wave components and IPLs, especially I-III, I-V than the normal controls.

- Usha Panjwani et al[83] studied the effects of antiepileptic drugs on BAEPs in 32 female epileptic patients. They observed that drug free epileptics had shortened wave V absolute latency and I-V IPL as compared to normal controls.

The development in the field of recording evoked potentials is closely linked to the discovery of electricity.

1752-Benjamin Franklin with his kite experiment charged his leyden jar by using kite during electrical storms and postulated the presence of two opposing forces of electricity that is positive and negative.

1791- Luigi Galvani discovered that nerves were good conductors of electricity.

1850-Helmholtz was able to measure conduction velocity of nerve in frog.

1861- The method of electro diagnosis based on faradic and galvanic current was introduced by Erb.

1875- The credit of making the first observation of electrical activity of brain

goes to Richard Canton, who reported that he had detected currents from electrodes placed on the skull of exposed brain in rabbits and monkeys.

1929- Hans Berger recorded the first human electroencephalogram from the electrodes placed on scalp.

1939- Davis was the first to record electric potentials on the human skull in response to auditory stimuli. The potentials were generated in the cortex with latencies that ranged from 50 to 500 ms. Some years later, thanks to computers, faster and shorter amplitude responses were recorded called as middle latency potentials between 10-80 ms.

1967-Sohmer and Feimesser were the first to record BAEP initially and attributed their origin to brainstem structures[37].

1971-Jewett and Williston were the first to describe BAEP waveforms.

1974-Hecox and Galambos showed that ABR could be used for threshold estimation in adults and infants.

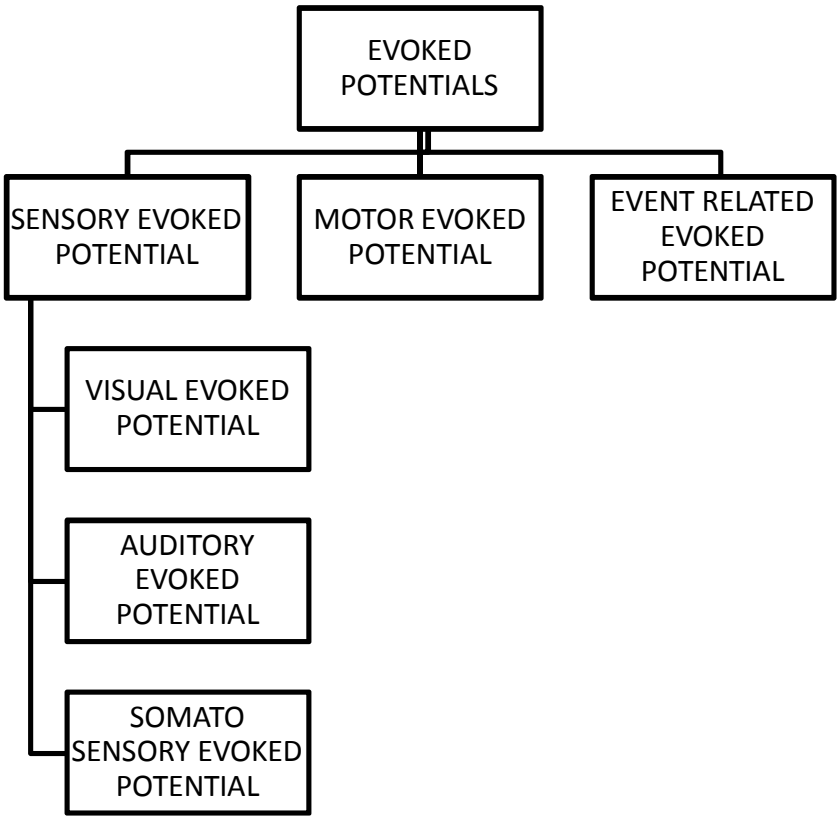
The clinical utility of evoked potentials is based on their ability to

- Demonstrate abnormal sensory system function when the history and /or neurological examination are equivocal.
- Reveal the presence of clinically unsuspected malfunction in a sensory system when demyelinating disease is suspected because of symptoms

and signs in another area of CNS.

- Help in defining the anatomic distribution of a disease process
- Monitor the changes objectively over time in a patient's status

FIGURE 3: Classification of evoked potentials



Sensory evoked potentials are recorded from CNS following stimulation of sensory organs. For example visual evoked potentials are elicited by a flashing light or changing patterns on a monitor; AEPs by a click stimulus presented through the ear phone and tactile or somatosensory evoked potential elicited by tactile or electrical stimulation of a sensory or

mixed nerve in the periphery. All these potentials are reliable diagnostic tests providing an objective measure of function in their related sensory system and tracts.

2.2.2. Brainstem Auditory Evoked Potential

The ground work for recording BAEP was laid in early 1930s when the electronic amplifiers became available. In 1939, the electrical response to auditory stimuli was first observed in raw EEG.

Recording BAEP is a simple non-invasive way of evaluating hearing function and has been widely used for early detection of neural conduction irregularities in the auditory pathway. Technical advances have lead to more widespread use of BAEP in various fields like audiology, neurology, anaesthesiology and neonatology.

Bluestone CD et al had acknowledged in their study that a normal ABR finding does not guarantee hearing and not all normally hearing subjects have normal ABR findings[40].

In spite of many advantages, BAEP equally has limitations- requires an experienced person to identify the waveforms accurately and the procedure is time consuming (Graham JM et al 2007)[41]. Development of sophisticated techniques like CT/MRI have limited the use of BAEP.

2.2.3. Principle of auditory evoked potential[35]

The evoked response audiometry is based on the principle that the bioelectric response which is evoked by a sound stimulus always tend to occur after the same time interval.

The auditory pathway extends from the middle ear structures through the eighth cranial nerve, the brain stem, and finally to the auditory cortex. Auditory stimuli either in the form of clicks or pure tones can be used to assess the integrity of the auditory pathway.

Thus the auditory evoked potential is obtained by presenting auditory stimuli to each ear resulting in a sequence of waveforms which bear a close relationship to the structures in the auditory pathway and enables relatively specific localisation in the auditory pathway, particularly in the eighth cranial nerve and the brainstem. From the time of onset of the sound stimulus, the auditory evoked transient response can be recorded up to 500 milliseconds.

2.2.4. Classification of Brainstem auditory evoked potentials

This tool of investigation which was first described by Jewett and Williston in 1971 can be classified as short, middle and long latency responses.

Short latency response

The normal Brainstem auditory evoked potentials occurring within first 10 ms give unique information about the brainstem functions is called the early phase of transient response or short latency response. These potentials are well known as Brainstem Evoked Response Audiometry (BERA) or Brainstem Auditory Evoked Potentials (BAEP) and this has been utilised extensively by the clinicians.

Advantages of BAEP include

1. This test is used to detect deafness in uncooperative patients like infants and mentally retarded or malingering individuals and can also be carried out correctly even in deeply sedated and anaesthetised patients.

2. Objectively determines the nature of deafness (i.e. sensory or neural) in difficult to test patients especially when they cannot respond adequately to tests.

3. Identification of the site of lesion in retro cochlear pathologies- The retro cochlear area is fairly a large area extending right from the spiral ganglion of the cochlear nerve to the midbrain (level of inferior colliculus). Unlike other tests used by the neurotologists which merely suspect whether any retro cochlear disease is present or not, BAEP

helps to identify the approximate area in the retro cochlear pathway where the lesion is present which is utilised in diagnosing conditions like acoustic neuroma with accuracy.

4. Study of central auditory disorders- Evoked response audiometry is useful in differentiating diseases of the auditory cortex from diseases of the more peripheral organs.

5. Helps to analyse the maturity of the central nervous system especially in new born, evaluating prognosis in comatosed patients, objective identification of brain death etc.

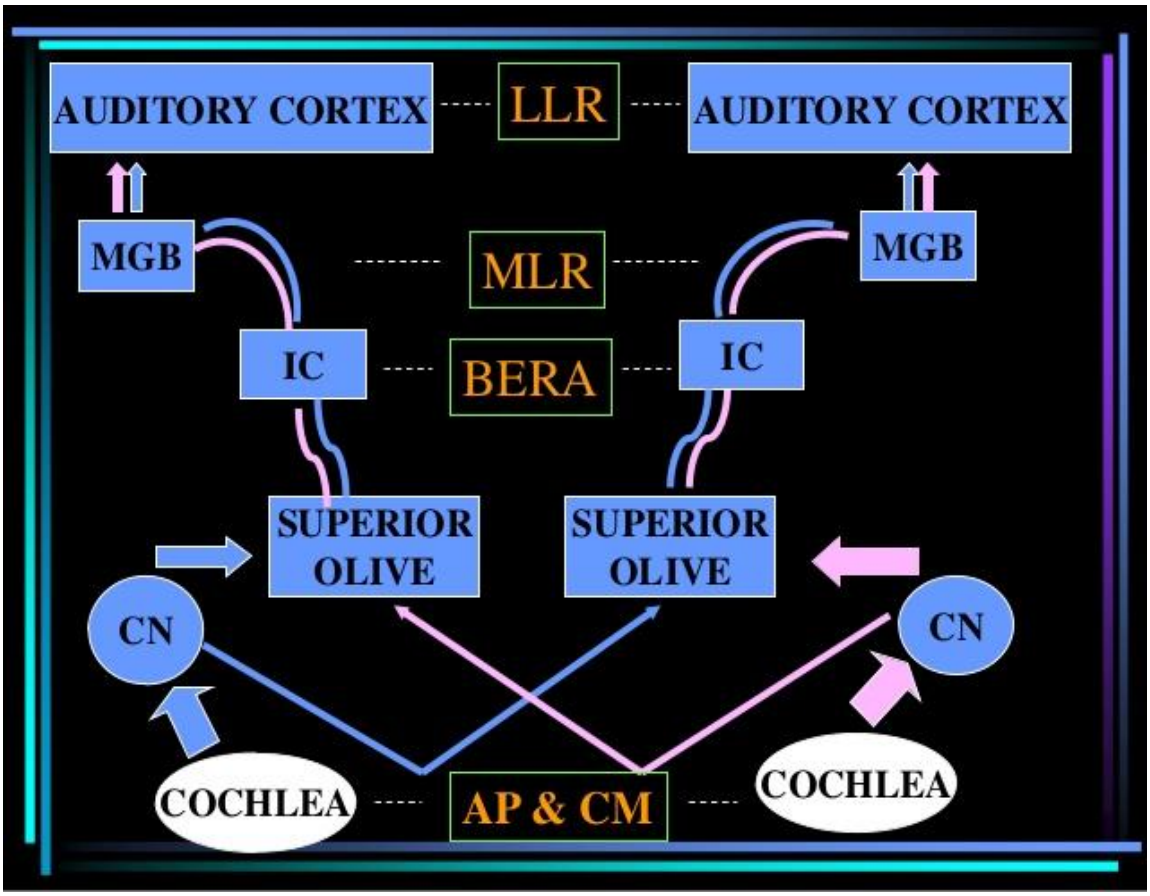
Middle latency response (MLR)

Auditory evoked potentials occurring before 10-50 msec is called MLR which reflects the activation of both subcortical structures(thalamus) and auditory cortices(mainly primary auditory cortex)[42].

Long latency response (LLR)

This response arises from multiple cortical generator sources (Neshige et al1992)[43] and have latencies greater than 50 msec distributed entirely over the scalp.

FIGURE 4: Generators of auditory evoked potentials



LLR - Long latency response

MLR - Middle latency response

BERA - Brainstem evoked response audiometry

AP - Action potential

CM - Cochlear microphonics

MGB - Medial geniculate body

IC -Inferior colliculus

2.2.5. Anatomical and Neurophysiological basis of brainstem auditory evoked response audiometry[37]

The sound pressure waves causes displacement of tympanic membrane which is transmitted through inner ear ossicles to the oval window. This causes movement of perilymph present in the scala vestibuli and tympani and secondarily of potassium rich endolymph contained in the ductus cochlearis and hence the basilar membrane, spiral organ and tectorial membrane are displaced.

So the spiral organ containing the hair cells produce auditory receptor potentials when it is displaced by the movement of tectorial membrane. The receptor potentials tend to trigger action potentials in the dendrites of afferent nerve fibres of cochlear nerve due to the release of neurotransmitters. It is approximately estimated that 20000 to 30000 hair cells are scattered over a distance of 31.5mm in 2.5 spirals of cochlea. It is found that low frequency sounds activate the apical portion of cochlea and high frequency sounds generate receptor potentials in the basal portion. The click stimulus used in BERA contains mainly high frequency tones which stimulates the basal portion of cochlea.

The cochlear nerve neurons situated in the spiral ganglia are bipolar with their dendrites in the hair cells and axons reaching the cochlear nucleus.

This cochlear nucleus has 3 sub nuclei components:

1. Anterior ventral cochlear nucleus (AVCN)

2. Posterior ventral cochlear nucleus (PCVN)

3. Dorsal cochlear nucleus (DCN)

The output of AVCN runs through the ventral acoustic striae forming the bulk of trapezoid body and terminates in the superior olivary nucleus and inferior colliculus. The neurons in AVCN tend to discharge at short latency to acoustic stimuli with a pattern similar to that of cochlear nerve.

Most of the output of PVCN goes through the ventral and middle acoustic striae to terminate in the superior olivary nucleus and inferior colliculus. Through the dorsal striae, the dorsal cochlear nucleus terminates in the superior olivary nucleus and contralateral inferior nucleus. The discharge from these neurons have a longer latency thus differing from AVCN.

The cochlear nucleus thus terminates in the superior olivary nuclear complex which includes 2 components-medial and lateral at the base of pons. The medial superior olivary nucleus receives excitatory inputs from both ipsilateral and contralateral AVCN. The lateral superior olivary nucleus also receives excitatory inputs from both ipsilateral AVCN and PVCN as well as

inhibitory inputs from contralateral AVCN and PVCN via) trapezoid body. From the olivary nucleus, the impulses travel to the ipsilateral and contralateral lateral lemniscus and to inferior colliculi. The olivary nuclei are the first site in the auditory pathways where the neurons are affected in a nonlinear manner to binaural stimulation.

The lateral lemniscus nuclei and inferior colliculi converges the input from contralateral cochlear nucleus and superior olivary nucleus. The impulse from the inferior colliculi reaches the medial geniculate body where the neurons form the acoustic radiation of internal capsule finally synapsing in the Heschl gyrus of the primary auditory cortex (superior temporal gyrus and upper bank of sylvian fissure including the frontal and parietal opercula), the deeper mesial portion of which is activated by the high frequency tones like clicks used in BERA.

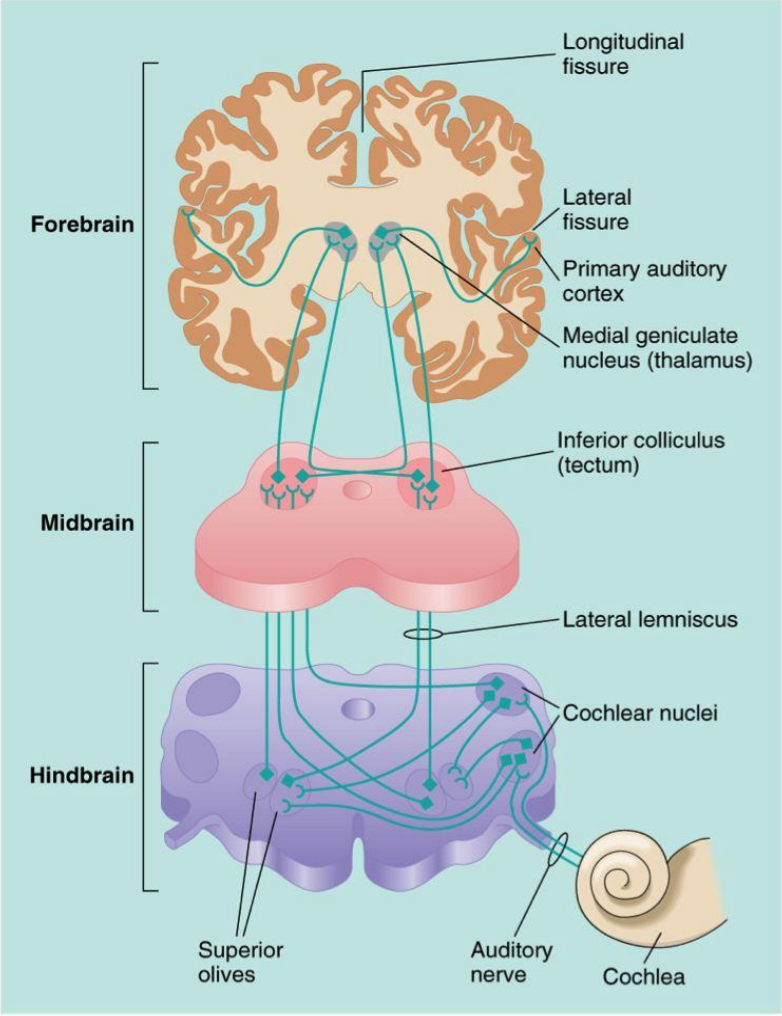
The orderly orientation of the neurons in dorsal cochlear, medial superior olivary and lateral superior olivary nucleus results in summation of synaptic potentials to result in high amplitude electric fields.

The journey of the auditory impulses through this pathway generates an electric activity which can be recorded by placing surface electrodes on the scalp. This electrical activity is manifested as waveforms with discrete peaks in the BERA readings, the nature and character of which can be studied using

various parameters and this reveals the structural and functional integrity of the auditory pathway.

Spiral ganglion in the cochlea →Ventral and dorsal cochlear nuclei in the brainstem →Superior olivary complex in the midbrain →Lateral lemniscus in midbrain →Inferior colliculus in midbrain→Medial geniculate body in thalamus →Auditory area in cortex.

FIGURE 5: Auditory pathway



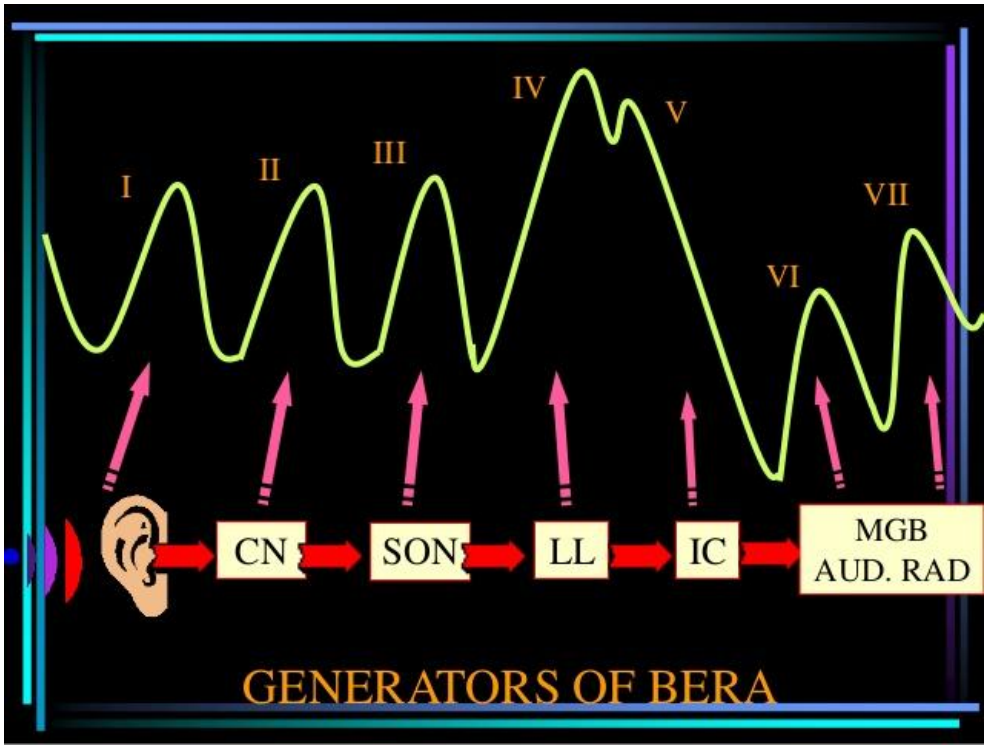
2.2.6. Normal Waveforms of BAEP

There are 5 or more distinct waveforms recorded within 10millisec of auditory stimuli (Jewett DL et al 1971)[44]. The analysis of these waveforms with regard to latency, amplitude, wave morphology provides neurodiagnostic information on cochlear and retrocochlear auditory function.

TABLE 1: Generators of BAEP waveforms

WAVEFORM	GENERATORS
I	Eighth nerve(Cochlear nerve)
II	Cochlear nucleus
III	Superior olivary nucleus
IV	Lateral lemniscus
V	Inferior colliculi

FIGURE 6: GENERATORS OF BAEP WAVEFORMS



2.2.7. Clinical applications

- Neil Bhattacharya[53] demonstrated that these evoked potentials can be utilised as an effective screening tool in the evaluation of suspected retro cochlear pathology such as vestibular schwannoma or acoustic neuroma.

- Young G Bryan[54] suggested that persistent abnormalities of BAEPs reliably indicate the likelihood permanent vegetative state or death.

- J.K.Nousak et al[55] showed that the BAEP latencies are accurate in evaluating hearing threshold.

- Studies carried out by Avasthi R Subhendu[56] have identified an increase in absolute latencies of all the waves of BAEP in patients with advanced hypertension and following treatment significant decrease in the wave latencies were observed demonstrating their role in prognostic follow up of these patients.

- Kurita A et al[57] compared 20 normal controls with diabetic patients and showed that diabetics had significantly longer latencies.

- Flint Boettcher A[58] demonstrated that latencies were prolonged in presbyacusis and can be used as an evaluating tool in elderly individuals for early detection of hearing loss.

- In a study conducted by Ikuta et al[59], he showed that differences in waveforms are seen in evoked potentials of schizophrenics, manic depressives and epileptics as compared to healthy adults.

- Atis et al[60] recorded BAEP in patients with COPD and attributed the prolonged latencies to chronic hypoxic-hypercapnic status occurring in the brainstem.

- Schwarz G et al[61] studied BAEP in patients with respiratory insufficiency following encephalitis and observed prolongation of all waves and IPL due to proximity of respiratory control centre in the brainstem.

- In a study done by Reyes Contreras et al[62], he observed significant differences in I-V IPL in HIV infected patients as compared with controls and concluded that HIV infection may produce subclinical pathologic changes in the cochlear nerve and brainstem which can be recorded by BAEP recordings.

- Leocani et al[63] in his study suggested that patients with multiple sclerosis can have abnormal ABRs.

- Lew and Henry L[64] demonstrated that BAEP recording can serve as an objective tool for estimating hearing dysfunction in traumatic brain injury patients.

2.3. Cytokines

They are low molecular weight regulatory proteins secreted by white blood cells and various other cells of the body in response to multiple stimuli[65]. In recent years an increasing body of evidence suggests that there is a complex relationship existing between epilepsy and immune system.

Plata –Salaman CR[66] observed abnormalities in the expression of cytokines and immune cells in patients with epilepsy.

Kalueff AV et al[67] recognised that the immune system and its associated inflammatory reactions play an important role in the process of epileptogenesis.

Steffensen SC et al[68] implicated cytokines as mediators of spontaneous seizures.

Fann MJ et al[69] identified that these cytokines influence many central neurotransmitters including Noradrenaline, Gamma amino butyric acid, Acetyl choline, 5 Hydroxy tryptamine as well as the expression of various neuropeptides in several brain regions.

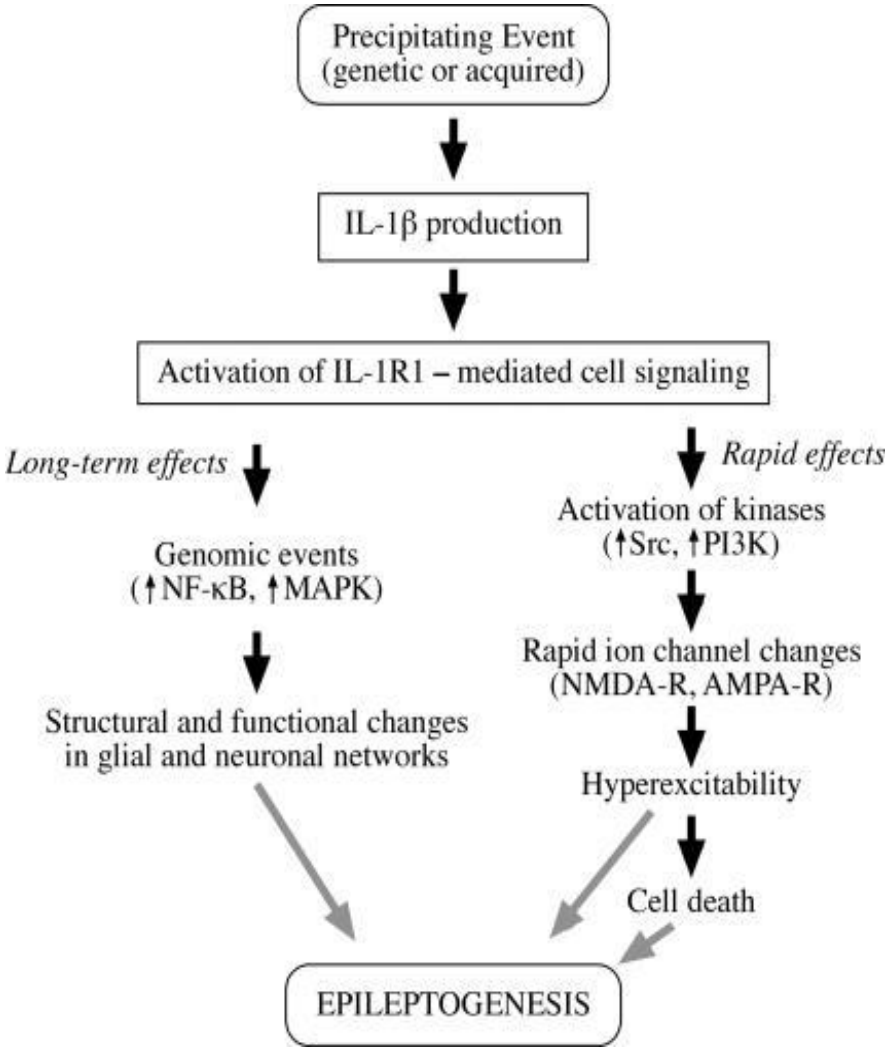
2.3.1. Interleukin-1beta (IL-1β)

IL -1β which is synonymous with catabolin is a cytokine protein encoded by the IL -β gene found on chromosome 2 in humans. IL- 1beta is a

pro inflammatory cyokine that activate additional cytokine cascade and enhances the susceptibility of seizures. IL 1 cytokines are regularly expressed at very low levels in human CNS (Ravizza T et al 2006)[70]. Seizures enhance the expression of IL- 1βand its mRNA as well as IL 1Ra mRNA (De Simoni MG et al 2000)[71]. Concentration of IL-1β in extracellular compartment is the prime factor for determining it functional actions in the brain.

IL 1β augment nitric oxide formation to raise the seizure susceptibility and also increase the neuronal excitability by directly inhibiting GABA(A)receptors, enhancing NMDA receptor function and inhibiting K efflux[104]. Viviani et al[105] has shown that IL-1β increases the phosphorylation of the NR2B subunit of NMDA receptor thereby enhancing Calcium influx into the neurons. Through the activation of sphingomyelinase, IL-1β induces the production of ceramide which in turn activates the Src family tyrosine kinases leading to NR2B phosphorylation. Balosso et al[106] suggest that the activation of this pathway underlies the proconvulsant activity of IL-1beta.

FIGURE 7: Interleukin-1beta in epileptogenesis



During epileptogenesis, strong IL-1β and IL-1R immunoreactivity was found also in perivascular astrocytic end feet impinging on blood vessels and in endothelial cells of microvasculature. Ravizza et al 2008 associated these changes with tissue extravasation of serum albumen. IL-1β can affect the permeability properties of blood brain barrier by disruption of tight junctions or nitric oxide production along with activation of metalloproteinases in endothelial cells which result in chronic neuronal hyperexcitability. Further

alterations in BBB permeability may favour the entry of the cells of adaptive and innate immunity into the brain which perpetuates inflammation.

Van illet et al 2006 proved that the extent of BBB damage positively correlates with the frequency of spontaneous seizures

IL-1β is cleaved from precursor protein (pro IL-1β) by IL-1β converting enzyme (ICE) otherwise called as caspase 1. Black et al showed that this cleavage is essential for the formation of active form of IL-1β. Recently Ravizza et al has reported a novel anticonvulsant treatment strategy which inhibits IL-1β production in brain using ICE inhibitors resulting in reduced seizure duration as well as increased resistance to seizures in kainic acid models.

Peltola et al[92] suggests that the levels of various cytokines increase transiently in the blood and CSF of patients with epilepsy after different types of seizure and the cytokine concentration was found to higher in CSF than in blood suggesting a brain origin

Vezzani et al in his experimental studies identified that IL-1β prolong the duration of kainic acid induced seizures.

Rosenbaum KJ et al[129] acknowledged that seizures themselves can activate the sympathetic nervous system and induce the release of catecholamine which mediates cytokine release from the peripheral blood mononuclear cells.

Lehtimaki et al[101] have proved that that the levels of IL 1 beta, IL1ra and IL6 were transiently elevated after electrographic seizures.

Gang Li et al suggests that the level of IL-1β, IL-6 and TNF alpha increases quickly after either GTCS or complex partial seizures and return to baseline after varying time intervals.

S.Sinha, S.A.Patil, V.Jeyalekshmy et al[91] analysed serum cytokine levels in 100 patients with epilepsy and new onset seizure in the immediate postictal phase. They observed a highly significant increase in serum levels of IL-1β, IL-2, 4, 6, TNF-alpha, IFN gamma in epilepsy patients as compared to the controls.

3. AIM & OBJECTIVES

3. AIM AND OBJECTIVES

The aim of this study is to evaluate the Brainstem auditory evoked potential in patients with Generalised Tonic Clonic Seizures in comparison with age and sex matched controls.

The objectives of the study were

- To determine the functional integrity of auditory pathway in patients with GTCS by recording brainstem auditory evoked potential.
- To assess serum Interleukin -1 beta levels in these patients.
- To find the correlation between serum Interleukin-1 beta level and Brainstem auditory evoked potential in patients with Generalized Tonic Clonic Seizures.

4. MATERIALS

& METHODS

4. MATERIALS AND METHODS

The study was conducted during the year 2013-2014 in the Institute of Physiology and Experimental Medicine, Madras Medical College after obtaining approval from the Institutional Ethics Committee, Madras Medical College Chennai.

4.1 Patient selection

Patients of both sexes in the age group between 20-40 years diagnosed as generalized tonic clonic seizures were included in the study. They were selected from the Institute of Neurology, Rajiv Gandhi Government General Hospital, Chennai - 3. 30 age and sex matched apparently healthy people were selected as controls.

4.2 Inclusion criteria

Thirty patients , both men and women in the age group of 20-40 years diagnosed as generalized tonic clonic seizures who were on treatment were included in the study after confirming the normal hearing ability of these persons using pure tone audiogram.

4.3 Exclusion criteria

- Children and pregnant women
- Patients with diabetes and hypertension
- Subjects with congenital hearing loss and sensorineural deafness
- Tumours like acoustic neuroma and meningioma
- Acute brainstem stroke
- Demyelinating diseases like multiple sclerosis
- Subjects with head injury and infections like meningitis and encephalitis
- Neurodegenerative diseases like dementia
- Febrile seizures
- Conditions that mimic GTCS like psychogenic nonepileptiform seizures
- Subjects with neoplastic, hepatic, respiratory and any cardiovascular disorder or other concurrent medical illness

4.4 Control group

Thirty age and sex matched controls were selected from technicians, staffs and attenders of the patients.

With all these criteria, a total of 60 individuals were selected for the study. Out of these 30 were apparently normal and termed as controls and the remaining 30 persons were patients with GTCS who were called cases. Informed verbal and written consent was obtained from the participants after explaining the procedure.

STUDY DESIGN: Cross sectional study

TYPE OF STUDY: Comparative study

PLACE OF STUDY: Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai.

All subjects included in the study had no hearing deficit as reported after thorough ENT examination which includes pure tone audiometry.

Specific ENT examination

Both the control and study group of individuals were subjected for specific ENT examination in the Upgraded Institute of Otorhinolaryngology, Madras Medical College, Chennai.

The ENT examination comprises of external ear examination, tuning fork tests which include Rinne's test and Weber's test, otoscopic examination of tympanic membrane, presence of any obstruction by wax and examination of throat and nose. In the presence of any external ear obstruction with wax, it is promptly removed by appropriate treatment before proceeding for pure tone audiometry.

Pure tone audiometry[72]

Both the normal controls and GTCS patients were subjected for pure tone audiometry in the Institute of ENT, Madras Medical College.

This procedure is performed to

i. Find out the hearing threshold of the subjects

ii. To rule out any external or middle ear pathology (the integrity of conducting pathway

Principle and method of pure tone audiometry

This is the most widely used method for measuring hearing acuity. A pure tone audiometer is an instrument that can deliver tones of variable frequencies and intensities to the ear by means of an earphone. The frequencies which are usually tested are at octave steps- 125,250,500,1000,2000,4000 and 8000 Hz. The intensity can be increased or

decreased for each frequency and that can vary from 10 dB to 120 dB. Most audiometers used nowadays are calibrated to the international (ISO) standard level to obtain accurate results.

Both air conduction and bone conduction can be measured by using this instrument. The ideal frequency to start with 1000 Hz, a series of tone pips or short signals are presented at intensity above the patients suspected threshold and the patient is instructed to signal every time whenever he hears a sound, the intensity is reduced in steps of 10 dB until no sound is heard by the subject. Now again the intensity is increased in steps of 5 dB until half of the tonepips are consistently heard. From this the patient's threshold for that frequency can be determined and thresholds for the remaining frequencies are then measured. In a similar way the bone conduction is measured by putting a receiver onto the mastoid bone. The sound emitted is transmitted to the cochlea by the bones of skull, thus bypassing the external and middle ear and this gives a measure of inner ear function. The results are interpreted as audiograms.

In audiometry, it becomes essential to eliminate the possibility that the test sound can be heard in the opposite ear for which masking should be applied to the better ear while testing the diseased ear if the difference in threshold is 40 dB or more. Similarly when testing the bone conduction threshold, the other

ear should always be masked because of the possibility of sound transmission through the bones of the skull.

4.5 Brainstem Auditory Evoked Potential

Both the group of subjects (normal controls and patients with GTCS) were subjected to the noninvasive assessment of hearing i.e.) Brainstem Auditory Evoked Potential (BAEP).

4.6 Apparatus for BAEP

The apparatus setup for measuring Brainstem evoked response audiometry are set as per the "Recommended standards for the clinical practice of evoked potentials" introduced in Guideline 9A: Guidelines on evoked potential, by American society of Clinical Neurophysiology[73].

The basic apparatus for recording of auditory brainstem evoked responses is illustrated in the figure

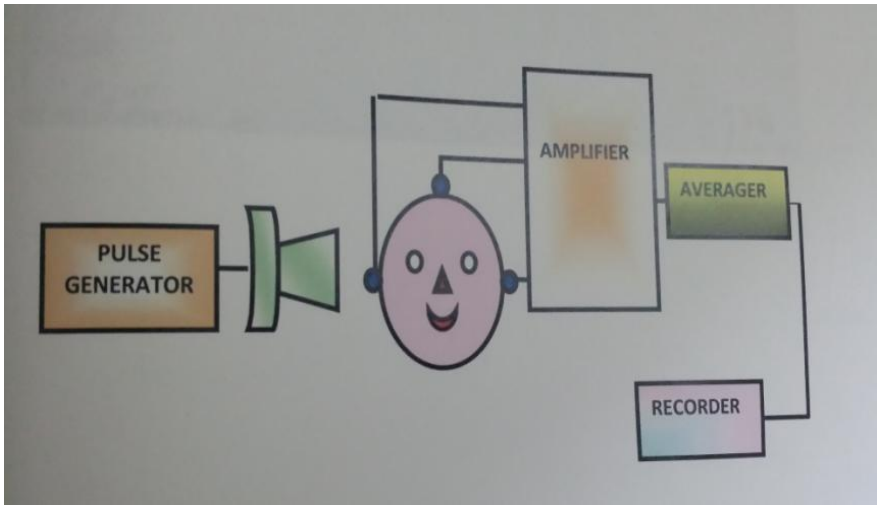


Figure 8: APPARATUS FOR BAEP

Photo No.1: Computerised Neurostim-Medicaid systems for recording Brainstem auditory evoked potential

Pulse generator

The stimulus which is either in the form of clicks or tone pips is transmitted to the ear through the transducer placed in the headphone or insert ear phone.

Recording electrodes

In recording auditory evoked potentials, three electrodes – active, reference and ground are used which are placed in the respective sites of scalp as per the International 10-20 electrode placement system. Both needle and surface electrodes can be used for recording AEP but ideally surface electrodes are preferred because it makes the procedure painless with lesser chances of infection.

For better placement of electrodes the patient should be instructed to have a shampoo bath to make the hair oil free before coming for the investigation. 1 cm disc electrodes filled with conducing jelly or paste are used. The electrical impedance should be kept below 5 kilo ohms for better recording of AEP, if it is too high the skin should be again cleansed with acetone and the surface electrodes should be reapplied with electrolyte jelly or EEG paste.

The electrode on the vertex serves as the reference whereas active and

ground electrodes are placed on the ipsilateral and contralateral mastoid process respectively.

Filter

Filter is a device that restricts selectively the frequency domain of the signal. The filter band pass is the frequency range of a signal which is transmitted through the filter. The frequency range in which a signal is rejected is called stop band. Between the pass band and the stop band, lies the transition band which is the characteristic of the filter. Filtering of the neurophysiological signals is essential for noise elimination and optimizing the recording. It is also useful to bring out the typical characteristics of the wave forms, which may not be obvious otherwise.

The low frequency filters remove the slowly changing low frequency components and permit the higher frequencies to pass through, therefore they are also called high pass filters. Similarly the high frequency filters tend to eliminate the rapidly changing high frequency components and allow the low frequency to pass through hence they are otherwise called as low pass filters.

Amplifier

Because of the very small biological signals, a variable degree of amplification (upto 500000 times) is required equal to the range of Analog to digital converter. The electrode impedance includes intrinsic impedance of

the electrode and the impedance of electrode-skin interface. For the measurement of any electrical activity including action potential which is generated in CNS, nerve or muscle should flow through the electrode into the amplifier and return to the patient through the ground lead. Electrode impedance results in drop of the amplitude of the action potential. This attenuated action potential reaches the amplifiers.

In order to reduce this attenuation, the impedance of the amplifiers should be much greater than electrode impedance. The electrode and amplifier and amplifier impedance both are inversely related to frequency. A 100:1 ratio of electrode to amplifier impedance should be maintained across the range of frequencies contained in the waveform under study. This minimizes the distortion of waveforms and improves noise rejection. Unequal electrode impedance imbalances the electrode amplifier input, converting some of the noise into a different signal, which is amplified to the same extent as the neurophysiological signal.to reduce the impedance-induced noise, the active, reference and ground electrode impedance should be minimized.

Signal averager

The process of measuring the electrical activity of brain in response to sound stimuli given to the ear is a complicated manoeuver because some

amount of random and spontaneous electrical activity is continuously generated within the brain (also called background potential) and a recording of such electrical activity is termed as electroencephalography (EEG). So the electrical activity setup in the brain in response to the sound stimulus gets obscured by superimposing over the spontaneous electrical activity occurring in the brain.

The magnitude of the electrical activity evoked by a sound stimulus is comparatively small i.e.) 1/100 of that of spontaneous random electrical activity. To distinguish these two types of electrical activity, a process called signal averaging is performed. This is based on the fact that evoked electrical activity is time specific which occurs at a fixed or specific point of time after the sound stimulation whereas the random electrical activity occurs randomly and not time specific. It is because of this fact we arrive at a point that if the electrical activity generated by a very large number of separate sound stimuli at a specific point of time is added together, only the electrical activity evoked by the sound stimulus will keep on adding whereas the background potential which occurs at random without any time specificity will cancel each other. Thus this signal averaging technique enables to get the uncontaminated measure of the sound evoked electrical activity without amplifying the responses.

Photo No.2: Recording of Brainstem auditory evoked potential in a normal volunteer

Electrical safety

While recording BERA, care should be taken to ensure the safety of the patient. The grounding and chassis leakage current of all the instruments connected to the patient must be periodically checked and the equipment should be designed in such a way to prevent inadvertent shock during power on, power off and failures.

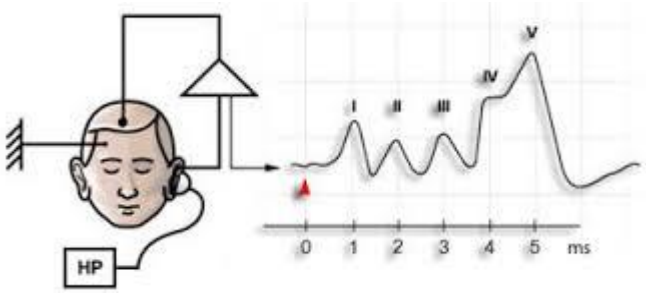
4.7 Procedure of recording BAEP

The subject is instructed to have a shampoo bath to make the hair oil free before coming for the investigation. The recording was carried out in a quiet and semi darkened room. The skin at the point of placement of electrodes was cleaned with spirit and cotton. Surface electrodes-active and ground are placed in the ipsilateral and contralateral mastoid processes respectively whereas the electrode on the vertex serve as the reference.

The resistance was kept below 5 kilo ohms. Monoaural auditory stimulus consisting of rarefaction clicks of 100 microsec were delivered through electrically shielded ear phones at the rate of 11.1 clicks/sec. The contralateral ear was suitably masked by pure white noise of 40dB thus preventing false BAEP response. A band pass of 150-3000 Hz was used to filter out undesirable frequencies in the surroundings. Responses to 2000 click presentations were averaged .

The result is obtained as a graph plotted with amplitude (in microvolts) on the ordinate and time (in ms from the onset of stimulus) on the abcissa. It consists of 5-7 waves or peaks within 8-10 ms and analysis of these waveforms with regard to latency, amplitude and morphology provides neurodiagnostic information on cochlear and retro cochlear function.

FIGURE 9 : Recording of BAEP waveforms



Wave I

This potential which is generated in the most peripheral portion of 8th CN corresponds to the compound AP recorded in electrocochleogram (Hashimoto I et al 1981)[45]. This prominent initial up going peak in the ipsilateral ear recording channel appears 1.4 ms after the stimulus and is attenuated or absent in the contralateral ear recording channel.

Wave II

This wave is poorly defined and appears as a small peak along the down going slope of wave I or in the up going slope of wave III. It comprises of 2 components

- APs in the intracranial portion of 8th CN which has been demonstrated by direct intra surgical recordings for the 8th nerve (Moller AR et al 1988)[46].

- Potentials generated in the intra parenchymal afferent auditory pathways in the ipsilateral rostral medulla which has been confirmed by animal studies showing that trapezoid body exhibits prominent activity at the timing of wave II (Caird D et al 1985)[47].

Wave III

This is usually a prominent peak which appears 3.9 ms after the click stimulus. Normal individuals may exhibit a bifid wave III in association with a normal I-V IPL.

Wave IV

This wave which occurs as a distinct and identifiable wave in 50-60% of the individuals is seen as a peak just preceding the wave V.

Wave V

It is the most prominent peak appearing 5.5 msec after the stimulus which is probably generated in the most lateral portion of lateral lemnisci or in inferior colliculi or in both. This wave component is analysed most often in clinical applications of ABR. In unilateral mesencephalic lesions, this wave is

consistently attenuated or even absent (Markand ON et al 1989)[48] during ipsilateral ear stimulation which has been demonstrated in many clinical studies.

Wave VI and VII

The origin of these waves are poorly defined and they are reasonably assumed to be generated from subcortical structures like MGB and auditory radiation with average latencies of 7.3 and 9.6 ms respectively. Depth electrodes placed in MGB show a positive peak at the time of wave VI.

4.8 Interpretation of waveforms

The parameters taken into consideration for studying the waveforms of BAEPs are

1. Absolute latency and amplitude
2. Inter peak latencies
3. Amplitude ratio of wave V/I
4. Inter ear inter peak difference

Absolute latency

Latency of a wave is the time interval which is measured in ms from the onset of stimulus to the peak of the wave. As absolute latencies are

affected by non-pathological factors, they are not reliable. The absolute latency of wave V is valuable for clinical purposes since it is the wave most commonly present and easily identifiable.

Absolute amplitude

This is measured as the height from the peak of the wave to its trough expressed in microvolts and their values are highly variable to be of any clinical significance because amplitude of the waves is not as constant as latency.

Inter peak latency (IPL)

The time interval between two different waves in the same ear is called inter peak latency which is otherwise called as inter wave latency. The IPLs commonly measured are

1. I-III IPL

It is represented by normal value of about 2.5 ms which reflects the conduction time between cochlea and the core of lower pons.

2. I-V IPL

This IPL is an index of the conduction time between the cochlea and proximal part of cochlear nerve and the midbrain through pons, the normal value of which is 4.5msec.

3. III-V IPL

The isolated prolongation of this IPL is not significant which measures the conduction from caudal pons to midbrain.

4. Amplitude ratio of wave V/I

This ratio compares the relationship of the signal amplitude since the wave I is generated outside and wave V is generated inside the CNS. The normal ratio varies between 50-100%. If the ratio exceeds 300%, it indicates peripheral hearing impairment but if it is less than 50%, then it implies significant central hearing loss.

5. Inter ear latency difference

When the same amount of supra threshold sound stimulus has been given to both the ears, it is the time interval between the 2 ears of the same wave which should not be more than 0.5msec.

TABLE 2: Normal waves of BAEP

WAVES (latency ms)	Misra and Kalita et al[37] n=30 pts ;15-68years	Chiappa et al[39] (1979)
I	1.67±0.17	1.7 ±0.15
II	2.78±0.21	2.8±0.17
III	3.65±0.22	3.9±0.19
IV	5.0±0.30	5.1±0.24
V	5.72±0.3	5.7±0.25
VI	7.2±0.48	7.3±0.29
I-III IPL	1.99±0.25	2.1±0.15
I-V IPL	2.08±0.30	1.9±0.18
III-V IPL	4.04±0.25	4.0±0.23

Factors that tend to affect BAEP are

1) Technical factors

A. Stimulus rate

The number of clicks presented to the ear in each second is defined as the stimulus rate. The normal recommended rate is 10-40 clicks /sec. As the stimulus rate becomes high, the amplitude and absolute latency of the waves show opposite changes i.e.) amplitude reduces whereas the latency increases.

B. Intensity of the sound stimulus.

At higher stimulation intensities, wave I often decreases at a slightly faster rate, resulting in relatively more prolonged I-V IPL. With decreasing click intensities all the waves except I, III, and V are likely to disappear. With even lower click intensities, only peak V may persist which may be seen with click intensities as low as 10 dBSL.

Also Elberling et al observed that the increase in wave V latency is not linear, with a relative acute change in latency occurring between 40 and 60dBSL clicks. This is one of the reason stimulation intensities of at least 70 dBSL are recommended for routine BERA studies in clinical practice.

C.Stimulus phase or polarity

The phase or polarity of the click stimulus can be of 2 types-condensation phase and rarefaction phase. The transducer of the BERA machine has got a diaphragm which vibrates i.e.) move outwards and inwards to produce the click sound.

When the diaphragm moves initially outward i.e.) towards the eardrum, the phase is termed as condensation phase whereas if it moves inward i.e.) in a direction away from the eardrum, it is known as rarefaction phase. This rarefaction phase is recommended for all routine BERA studies because of its capability to produce better resolution of the waves. Some

neurologists prefer using an alternating phase where rarefaction and condensation phases alternate closely with each other. If the condensation or alternating phase is used, the absolute latencies of all the waves I, II, III, IV are increased except wave V which is not that much altered by the phase change.

4. Filter characters of the machine

The BERA machine is adjusted in such a way that it records only a fixed range of frequencies which has a lower and higher limit. The lower frequency filter should be 100 or 150 Hz whereas the high frequency limit is usually kept at 3000 Hz and this is termed as filter settings of the machine.

Frequencies lower than 100 Hz and higher than 5000 Hz should be cut off to reduce the artefacts which may interfere with the BERA readings. So altering the filter settings can introduce artefacts and also change the amplitude ratios of the waves.

5. Nature of sound used

The sound stimulus used is a click sound produced by the transducer for eliciting auditory evoked potentials which is generated as a square wave pulse of 0.1msec duration each. The sound pressure wave which was generated by this stimulus can be displayed on an oscilloscope and consists of an initial major wave followed by highly damped oscillations of alternating

polarity that may last up to 2msec or longer. To obtain clearly recognisable and distinct waves, the sound stimuli are delivered at 50-60 db above the hearing threshold. Sometimes pure tone sound stimulus (tone pips) are used which presents with morphologically poor waves.

6. Binaural/monaural stimulation

Monaural stimulation is recommended in clinical studies but if the sound stimulus is presented to both ears simultaneously ,then the amplitude of the waves III, IV, V are increased but not wave I.

2) Nontechnical factors

A. Age

Age has a distinct effect on BAEP waveforms. Starr et al[49] observed longer BAEP wave latencies in infants when compared to adults which is ascribed to slower axonal conduction.

Jerger and Hall[50] experimentally noted these differences in 1980 when they examined the latencies in 70 normal subjects.

Stockard et al observed that the waveforms in infants are often higher in amplitude than in adults presumably on the basis of smaller head size and greater proximity of recording electrodes to BAEP generators.

Rowe et al[107] demonstrated in his study that selective prolongation of I-III IPL occurs in presbyacusis.

B. Gender

The difference in head size can be correlated to the significant difference of the wave components between males and females. The amplitude of all waves is found to be higher in a significant proportion of females whereas consistent prolongation of absolute latency of wave V and I-V IPL are noted in males.

TABLE 3: Gender differences in normal BAEP waveforms

Wave	**Taghavy &Losslein et al[51]**		**Pedialli et al 2004[52]**	
	Males	**Females**	**Males**	**Females**
I	1.64±0.16	1.59±0.09	1.42±0.06	1.44±0.10
II	2.83±0.19	2.75±0.12		
III	3.91±0.20	3.67±0.15	3.61± 0.13	3.59± 0.09
IV	5.14±0.25	4.97± 0.17		
V	5.80±0.20	5.52± 0.15	5.54± 0.21	5.32± 0.13
I-III	2.27± 0.20	2.08± 0.13	2.18±0.13	2.12±0.11
I-V	4.18±0.24	3.90±0.19	4.11±0.21	3.88±0.17
III-V	1.90±0.19	1.85±0.14	1.93±0.14	1.75±0.12

C.Temperature

With decreasing central body temperature, the latencies of BAEPs are increased. Latencies increase roughly 7% for each 1 Celsius. As the temperature reduces down to 27-28 C, the amplitude of BAEPs initially increases but then it drops down linearly with temperature drop and finally disappears at 20 degree C.

D. Hormonal effects

Yadav et al studied BAEP latencies in 20 women with normal cycles in 4 different phases and showed that there is a trend of increase in peak latencies of wave III and V in oestrogen-peak midcycle while decrease in latencies in progesterone-peak midluteal phase. These findings suggest that normal cyclical variations in the levels of oestrogen and progesterone during menstrual cycle do affect the auditory pathways and effects are better seen on the central component.

E.Drugs

BAEPs are resistant to the effect of drugs, but a slight prolongation of wave V latency with barbiturates or alcohol is attributed to the lowering of body temperature. Effects of aminoglycoside antibiotics on BAEP waveforms was studied with the idea of developing a means of following the ototoxicity of these drugs by Guerit et al in 1981 and they found that the

minor latency changes noted disappeared with cessation of medication.

4.9 Terminologies used in evoked potential study

1. Decibel (dB=1/10 Bel)

It is defined as ’20 log (P1/P2)’, where P1 is the intensity of the sound which is to be measured and P2 is the intensity of the reference sound.

2. Sound pressure level(spl):

It is the weakest sound heard by the most sensitive ear which is the standard physical reference for sound (20 micropascals or 0.0002 dynes per cm2).

3. Hearing level:

Zero dBHL corresponds to the average hearing threshold of a group of normal hearing young adults in an ideal listening environment.

4. Sensory level:

This expresses the intensity of a sound as a function of the hearing threshold for an individual ear for any given subject.

4.10 Parameters studied

BAEP threshold for each ear with absolute latencies of waves I, III, V and IPLs of I-III, I-V and III-V were considered from the recording for comparison among GTCS patients and controls.

4.11 Summary of BAEP settings in our study

1. APPARATUS: Neurostim, Medicaid systems

2. ELECTRICAL MONTAGE: L:Cz-A1

R:Cz-A2

Ground:Fz

3. AMPLIFIER: Low filter-100 Hz

High filter-3000 Hz

4. ANALYSIS TIME: 10 milliseconds

5. NUMBER OF EPOCS: 2000 trials with two repetitions

6. STIMULATION:

a. Nature of the stimulus to the test ear:

Broadband click of 100 micro seconds duration

Intensity -80 dB

Stimulus rate-11.1 clicks per second

b. Masking of contralateral ear: By white noise of 50-60 dB is used

4.12 Estimation of Serum Interleukin-1 beta

Under strict aseptic precautions, blood samples were collected from the ante cubital vein by means of venepuncture within 24 hours of the previous seizure episode and the separated serum was stored in deep freezer at -20 centigrade. Estimation of serum interleukin 1 beta levels was carried out in the Department of Experimental medicine, The Tamil Nadu Dr. M.G.R. Medical University.

Human IL-1β ELISA (Enzyme linked immunosorbent assay) kit is an in vitro enzyme linked immunosorbent assay for the quantitative measurement of IL-1β in serum, plasma and cell culture supernatants. The minimum detectable dose of IL-1β is typically less than 0.3 pg/ml.

Reagent preparation

1. Preparation of standard

Briefly spin the vial containing recombinant human IL- 1 beta to which 880microl of assay diluent A (0.09% sodium azide) is added to prepare a 20ng/ml standard. The powder is dissolved thoroughly by a gentle mix. Then 5 µl of IL-1 beta standard is added into a tube with 995 µl assay diluent A to prepare a 100 pg/ml stock standard solution. Use the standard stock solution for pipetting 200 microliter into each tube to produce a dilution series.

Photo No. 3: Interleukin-1β kit and serum samples, ELISA reader in TN Dr. M.G.R. Medical University

2. Wash concentrate(20x)

20 ml of wash buffer concentrate is diluted with distilled water to yield 400 ml of 1xwash buffer.

3. Detection antibody IL-1β (biotinylated anti human IL-1β)

100 µl of 1x assay diluent B (5x concentrated buffer) is added into the detection antibody vial to prepare a detection antibody concentrate which should be again diluted 80 fold with the same diluent.

4. HRP –streptavidin solution

HRP –streptavidin concentrate should be diluted 300 fold with 1x assay diluent B.

Assay procedure

This assay employs an antibody specific for human IL-1 beta coated on a 96 well plate.

1. 10 µl of each standard and sample are added into appropriate wells which is covered well and incubated for 2.5 hours at room temperature.
2. The solution is discarded and washed 4 times with 300microl of 1x wash buffer.

3. 100 µl of prepared biotinylated antibody is added to each well and incubated for 1 hour at room temperature with gentle shaking.

4. The solution is discarded and the wash procedure is repeated.

5. 100 µl of prepared streptavidin solution is added to each well and incubate for 45 min at room temperature.

6. Discard the solution and repeat the wash as described above.

7. 100 µl of TMB one step substrate reagent is added to each well and repeat the incubation for 30 min at room temperature in the dark.

8. Finally 50 µl of stop solution (0.2 M sulfuric acid) is added to each well and read at 450 nm immediately in a ELISA reader.

9. A standard graph is constructed by marking the average values of absorbance of each reference standard in the Y axis versus its corresponding concentration in X axis.

10. The corresponding IL- 1β was obtained by simple interpolation from this standard curve.

11. Average % was obtained by dividing observed value by expected value.

4.13 Statistical analysis

Statistical analysis was done using the software SPSS version 21.

1. Student 't' test was carried out to compare the means of variables between GTCS patients and normal subjects.

2. Pearson's coefficient was done to find the correlation between serum Interleukin-1β level and BERA in GTCS patients.

5. RESULTS

5. RESULTS

All the GTCS patients and controls enrolled for the present study had clinically no evidence of hearing deficit.

5.1 Characteristics of study and control subjects

Our study population consists of 30 GTCS patients (15 males and 15 females) in the age group 20-40 years without any clinical evidence of hearing impairment. The control subjects were 30 in number with 15 males and 15 females belonging to the age ranging from 20-40 years. The mean age was calculated to be 30.86±2.54 years in the control group and the mean age of GTCS patients was found to be 30.86±2.54 years. The duration of the disease ranges from 1-2 years.

5.2 Brainstem auditory evoked potential parameters

Variables pertaining to BERA between normal and GTCS patients are given in the tables 4-17 and also represented in graphs 1-10. Among the BERA parameters, the absolute latencies of wave I, III, V and IPL I-III, I-V, III-V are utilized for evaluating the integrity of auditory pathway.

5.3 Levels of Interleukin-1 beta

The difference in the mean values of IL-1β between controls and GTCS patients are given in table 18 and graph 11.

TABLE 4: Age distribution in controls and GTCS patients

	NUMBER	MEAN ±SD	ONE WAY ANOVA
GTCS FEMALES	15	30.2±4.79	F =2.34
CONTROLFEMALES	15	29.06±4.97	
GTCS MALES	15	32.33±2.63	
CONTROL MALES	15	32.66±4.62	
TOTAL	60		

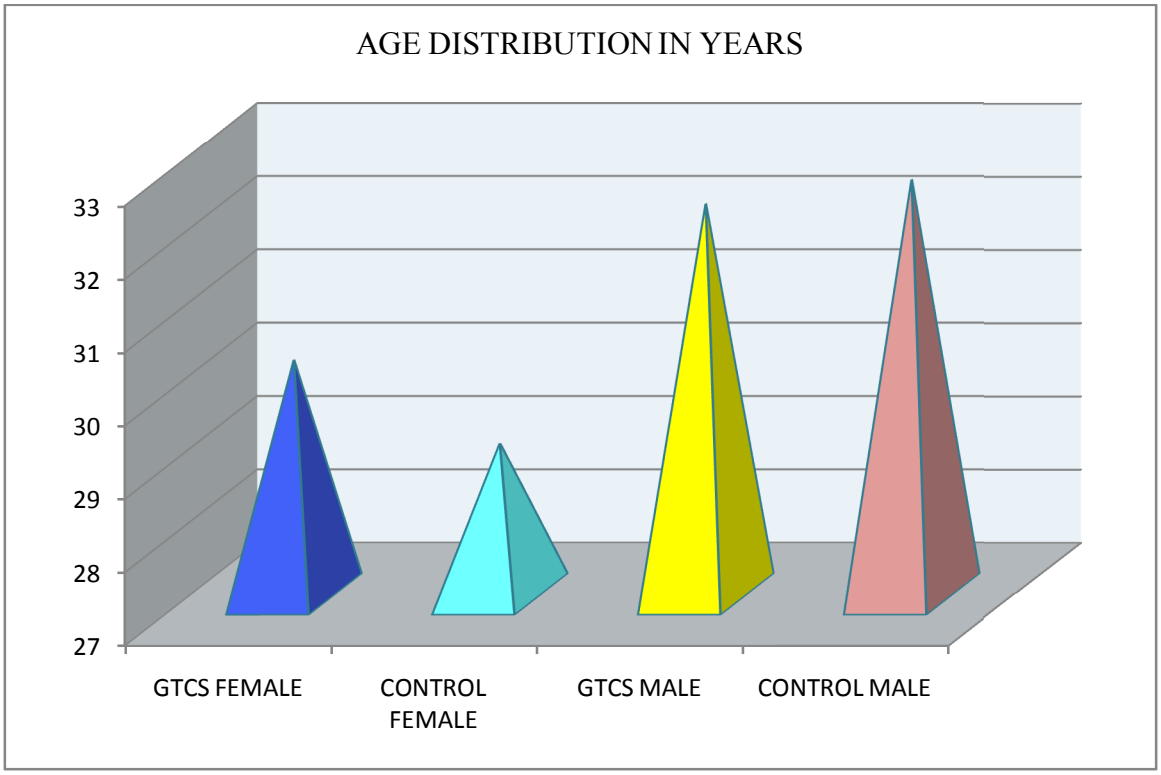
p value =0.082, not significant.

TABLE 5: BMI distribution in controls and GTCS patients

	NUMBER	MEAN ±SD	ONE WAY ANOVA
GTCS FEMALES	15	23.80±1.28	F =1.39
CONTROLFEMALES	15	23.98±0.83	
GTCS MALES	15	24.65±1.73	
CONTROL MALES	15	24.54±1.09	
TOTAL	60		

P value =0.25, not significant

GRAPH 1: Age distribution in controls and GTCS patients



GRAPH 2: DMI distribution in controls and GTCS patients

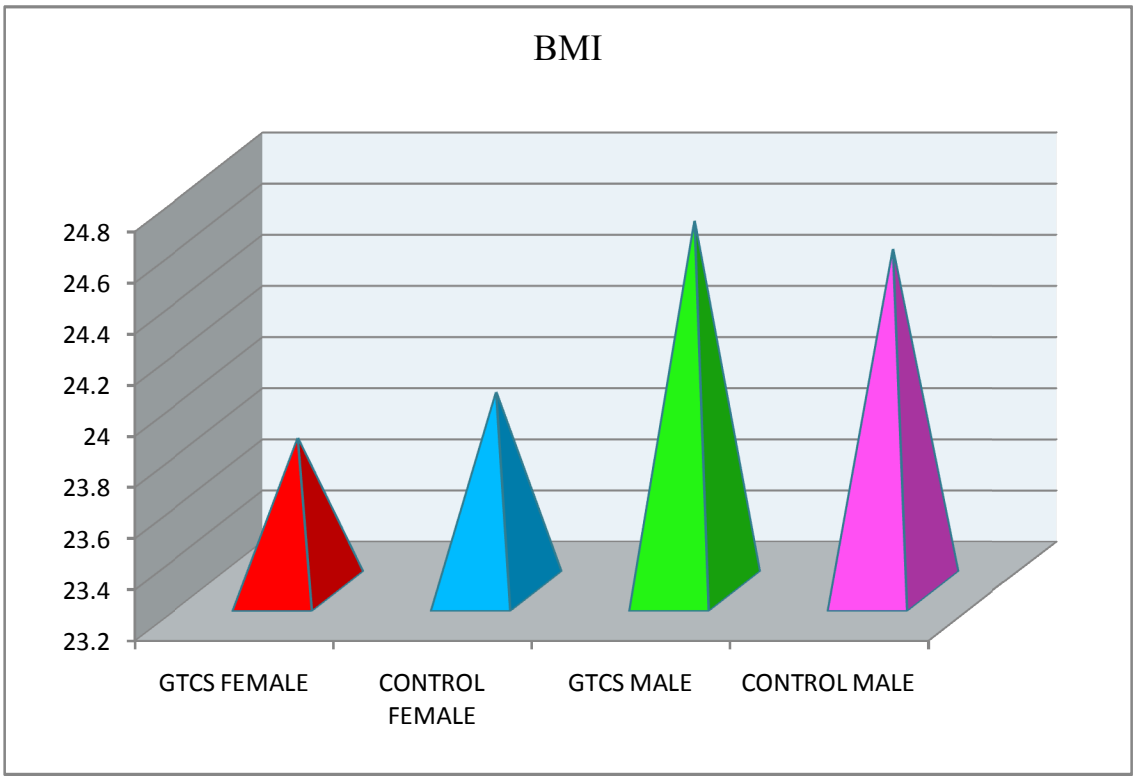


TABLE 6: Comparison of mean values of absolute and interpeak latencies between genders in the right ear of GTCS patients

WAVES	FEMALES	MALES	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I	1.60 ±0.21	1.61 ±0.15	0.15	0.88
III	3.85 ±0.23	3.76 ±0.30	1.51	0.14
V	5.67 ±0.17	5.59 ±0.19	1.21	0.23
I-III	2.25 ±0.31	2.14 ±0.35	0.91	0.37
I-V	4.07 ±0.17	3.97 ±0.27	1.21	0.23
III-V	1.82 ±0.25	1.83 ±0.20	0.12	0.90

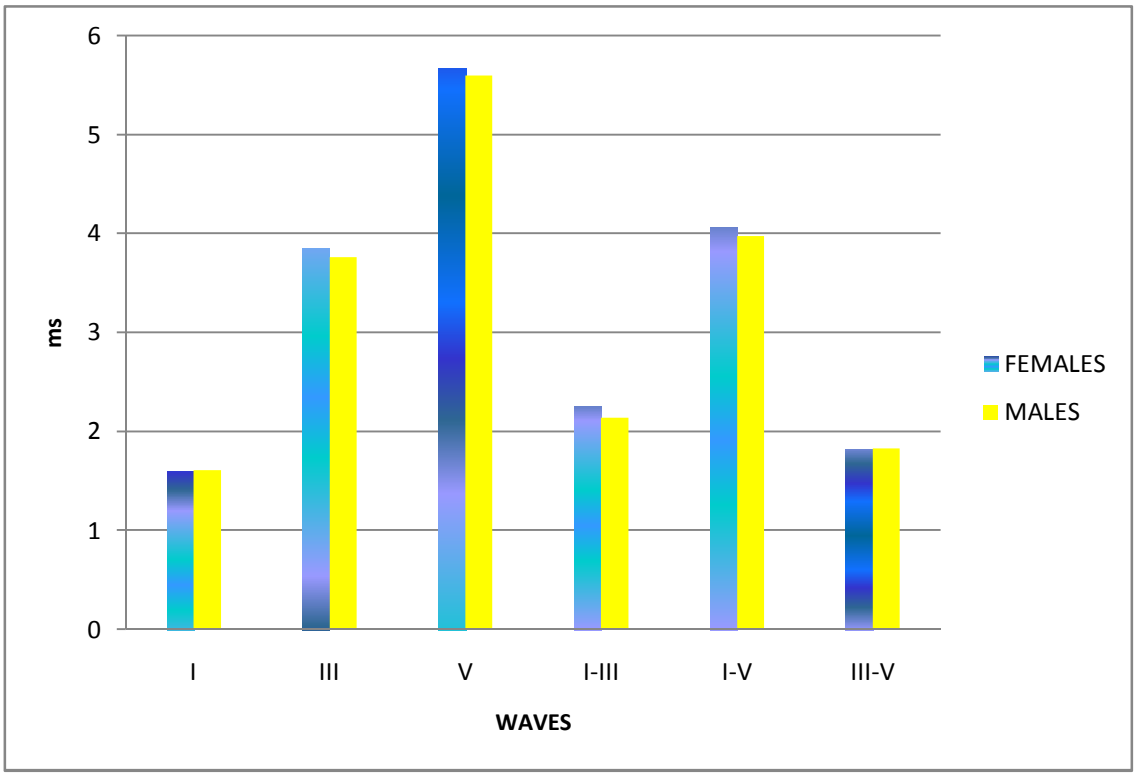
p value-not significant for all the parameters

TABLE 7: Comparison of mean values of absolute and interpeak latencies between genders in the left ear of GTCS patients

WAVES	FEMALES	MALES	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I	1.69 ±0.12	1.60 ±0.15	1.81	0.08
III	3.95 ±0.20	3.83 ±0.27	1.38	0.17
V	5.71 ±0.14	5.67 ±0.14	0.78	0.44
I-III	2.26 ±0.23	2.23 ±0.39	0.25	0.79
I-V	4.02 ±0.16	4.06 ±0.26	0.50	0.61
III-V	1.75 ±0.26	1.83 ±0.20	0.94	0.35

p value-not significant for all the parameters

GRAPH 3: Comparison of mean values of absolute and interpeak latencies between genders in the right ear of GTCS patients



GRAPH 4: Comparison of mean values of absolute and interpeak latencies between genders in the left ear of GTCS patients

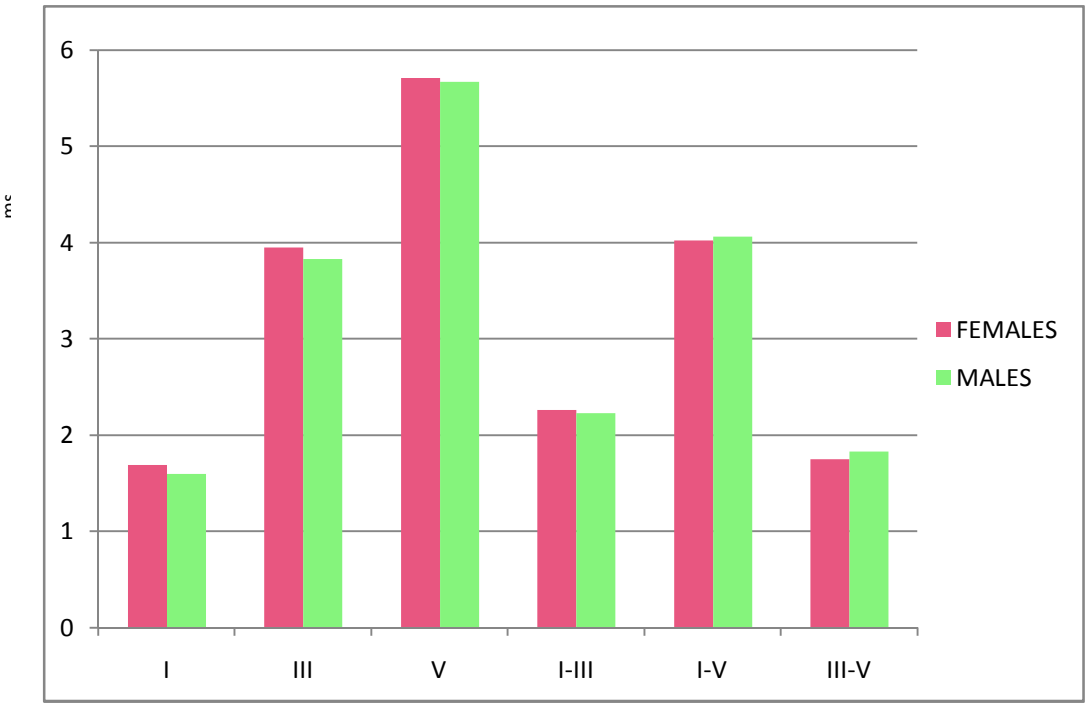


TABLE 8: Comparison of mean values of absolute and interpeak latencies between genders in the right ear of control group

WAVES	FEMALES	MALES	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I	1.64 ±0.14	1.70±0.20	0.96	0.34
III	3.67 ±0.14	3.72 ±0.18	1.38	0.17
V	5.47 ±0.21	5.64±0.19	2.30	0.02
I-III	2.03 ±0.19	1.96 ±0.26	0.84	0.40
I-V	3.83 ±0.27	3.89 ±0.22	0.66	0.51
III-V	1.81 ±0.25	1.89 ±0.14	1.08	0.29

Wave V, p value = 0.02, significant

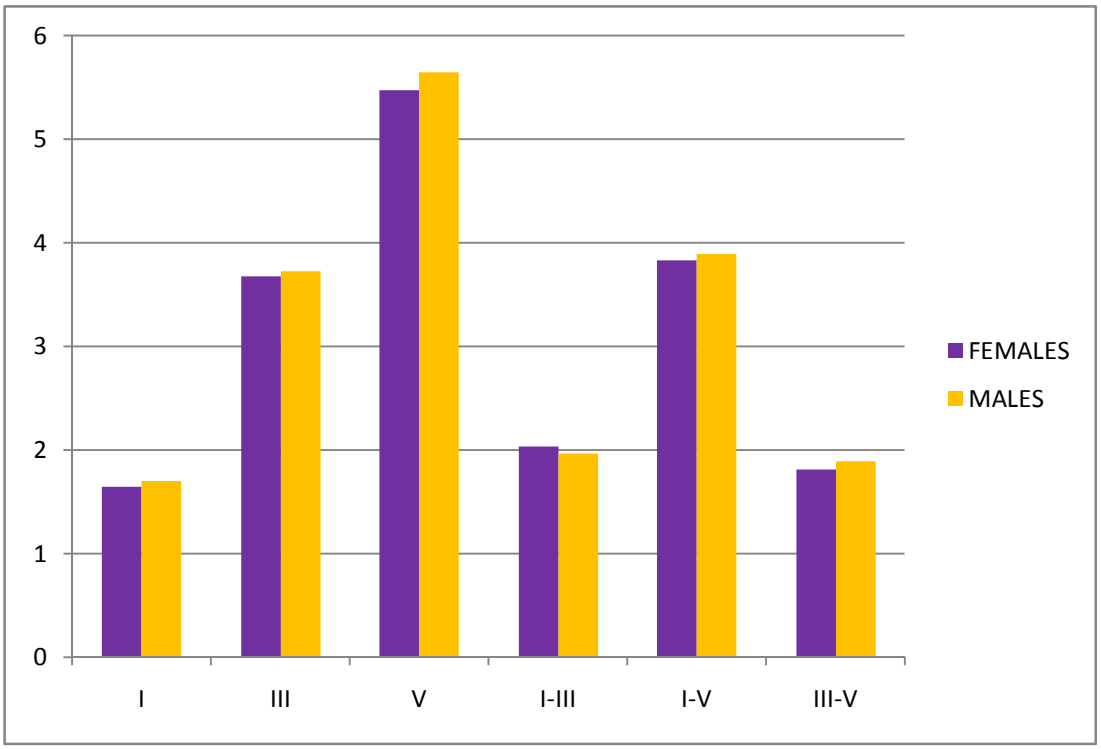
The mean value of wave V absolute latency is significantly higher in males as compared to females in the right ear of control group.

TABLE 9: Comparison of mean values of absolute and interpeak latencies between genders in the left ear of control group

WAVES	FEMALES	MALES	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I	1.65 ±0.16	1.69 ±0.19	0.62	0.53
III	3.68 ±0.22	3.70 ±0.23	0.24	0.81
V	5.46 ±0.26	5.59 ±0.25	1.39	0.17
I-III	2.03 ±0.25	2.02 ±0.15	0.13	0.89
I-V	3.80 ±0.33	3.89 ±0.33	0.74	0.46
III-V	1.77 ±0.29	1.89 ±0.36	1.005	0.32

p value –not significant for all the parameters

GRAPH 5: Comparison of mean values of absolute and interpeak latencies between genders in the right ear of control group



GRAPH 6: Comparison of mean values of absolute and interpeak latencies between genders in the left ear of control group

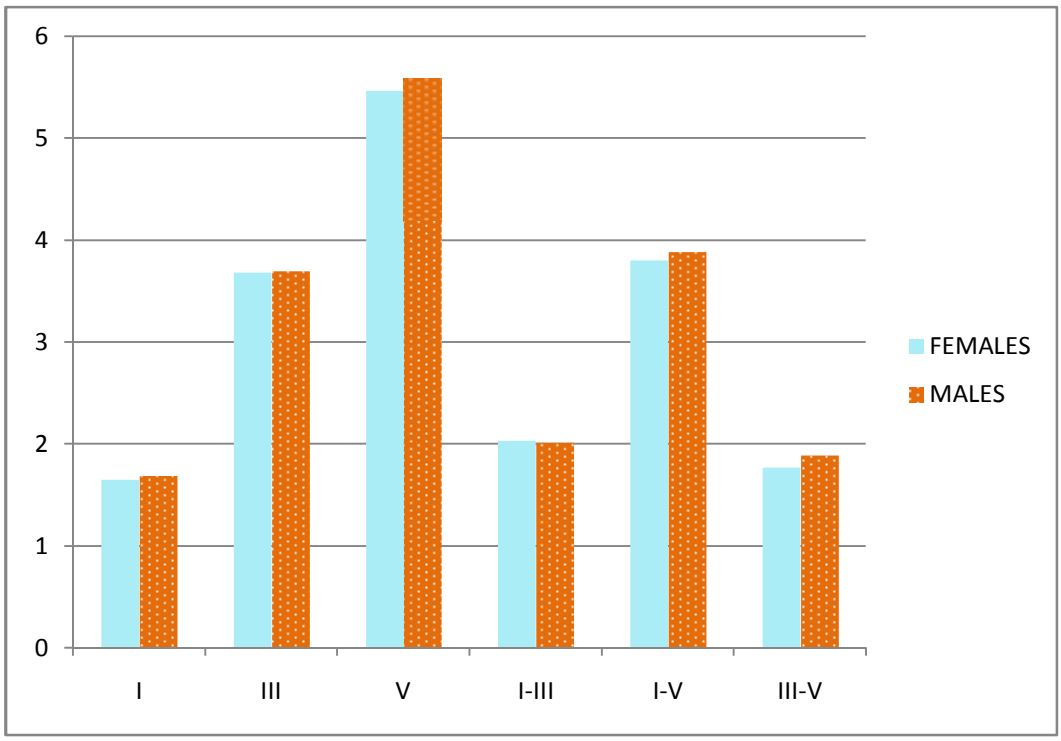


TABLE 10: Comparison of mean values of absolute latencies between GTCS females and control females in the right ear.

WAVES	STUDY GROUP	CONTROLGROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I	1.60 ±0.21	1.64 ±0.14	0.46	0.64
III	3.85 ±0.23	3.67 ±0.14	2.58	0.015
V	5.67 ±0.17	5.47 ±0.21	2.86	0.007

Wave III, p value=0.015, significant

Wave V, p value=0.007, highly significant

The mean values of wave III and V absolute latency are significantly higher in GTCS females when compared to control group.

TABLE 11: Comparison of mean values of interpeak latencies between GTCS females and control females in the right ear

IPL	STUDY GROUP	CONTROLGROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I-III	2.25 ±0.31	2.03 ±0.19	2.34	0.02
I-V	4.07 ±0.17	3.83 ±0.27	2.9	0.006
III-V	1.82 ±0.25	1.81 ±0.25	0.11	0.91

I-III IPL, p value=0.02, significant

I-V IPL, p value=0.006, significant

The mean values of IPL I-III and I-V are significantly higher in GTCS females when compared to control group

TABLE 12: Comparison of mean values of absolute latencies between GTCS females and control females in the left ear

WAVES	STUDYGROUP	CONTROLGROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I	1.69 ±0.12	1.65 ±0.16	0.77	0.44
III	3.95 ±0.20	3.68 ±0.22	3.51	0.001
V	5.71 ±0.14	5.46 ±0.26	3.27	0.002

Wave III, p value=0.001, highly significant

Wave V, p value=0.002, significant

The mean values of wave III and V absolute latencies are significantly higher in GTCS females as compared to control females.

TABLE 13: Comparison of mean values of interpeak latencies between GTCS females and control females in the left ear

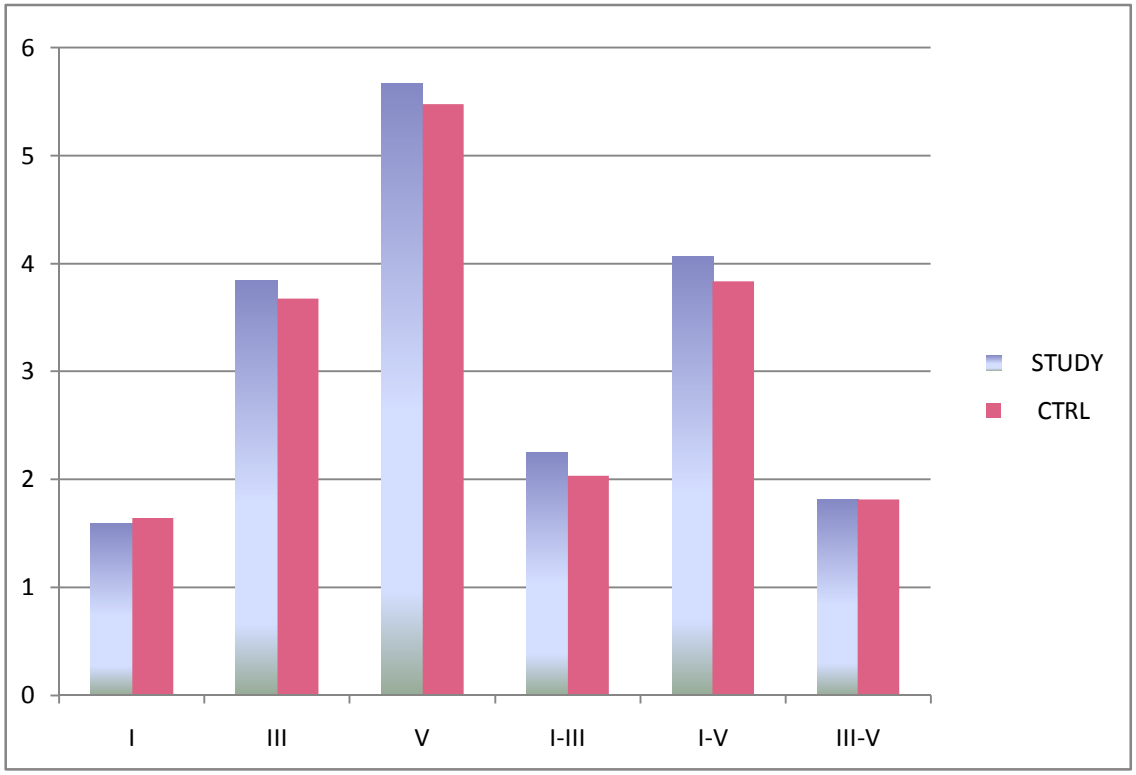
IPL	STUDYGROUP	CONTROLGROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I-III	2.26 ±0.23	2.03 ±0.25	2.62	0.01
I-V	4.02 ±0.16	3.80 ±0.33	2.32	0.02
III-V	1.75 ±0.26	1.77 ±0.29	0.19	0.84

I-III IPL, p value=0.01, highly significant

I-V IPL, p value=0.02, significant

The mean values of I-III and I-V IPL are significantly higher in GTCS females when compared to controls.

GRAPH 7: Comparison of mean values of absolute and interpeak latencies between GTCS females and control females in the right ear



GRAPH 8: Comparison of mean values of absolute and interpeak latencies between GTCS females and control females in the left ear

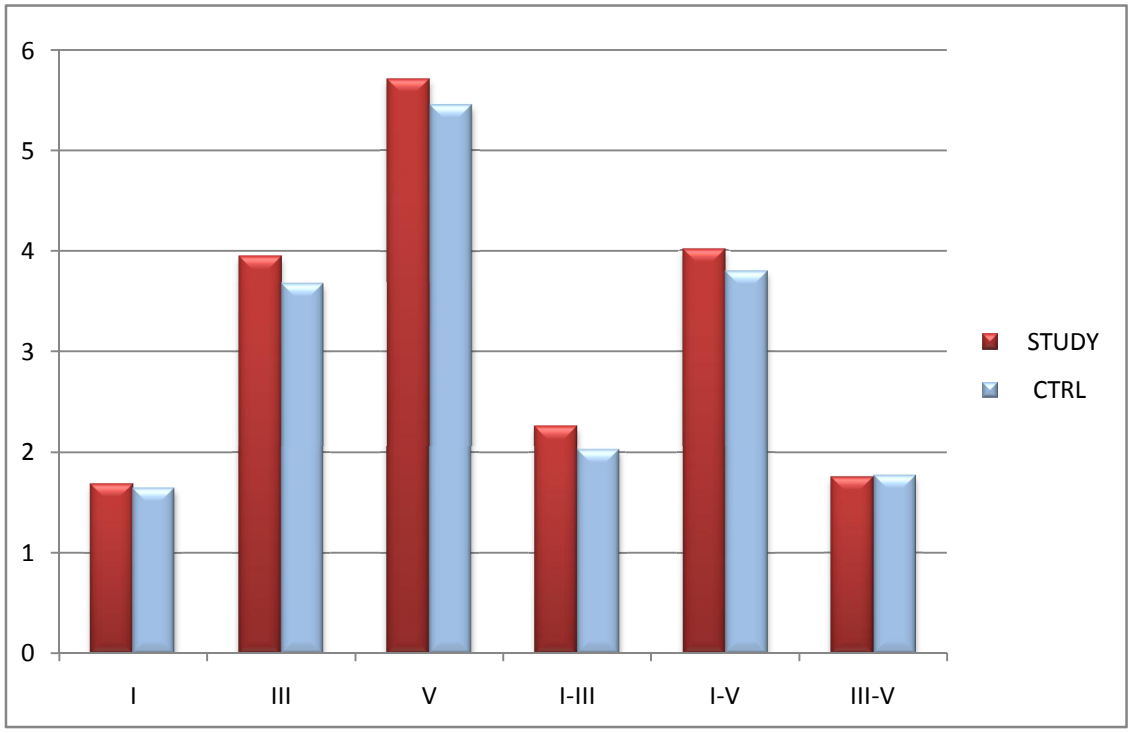


TABLE 14: Comparison of mean values of absolute latencies between GTCS males and control males in the right ear

WAVES	STUDY GROUP	CONTROLGROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I	1.61 ±0.15	1.70 ±0.20	1.39	0.17
III	3.76 ±0.30	3.72 ±0.18	0.44	0.66
V	5.59 ±0.19	5.64 ±0.19	0.72	0.47

p value – not significant for all parameters.

TABLE 15: Comparison of mean values of interpeak latencies between GTCS males and control males in the right ear

IPL	STUDY GROUP	CONTROLGROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I-III	2.14 ±0.35	1.96 ±0.26	1.59	0.12
I-V	3.97 ±0.27	3.89 ±0.22	0.88	0.38
III-V	1.83 ±0.20	1.89±0.14	0.95	0.34

p value – not significant for all parameters.

TABLE 16: Comparison of mean values of absolute latencies between GTCS males and control males in the left ear

WAVES	STUDY GROUP	CONTROL GROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I-III	2.23 ±0.39	2.02 ±0.15	1.94	0.06
I-V	4.06 ±0.26	3.89 ±0.33	1.56	0.12
III-V	1.83 ±0.20	1.89 ±0.36	0.56	0.57

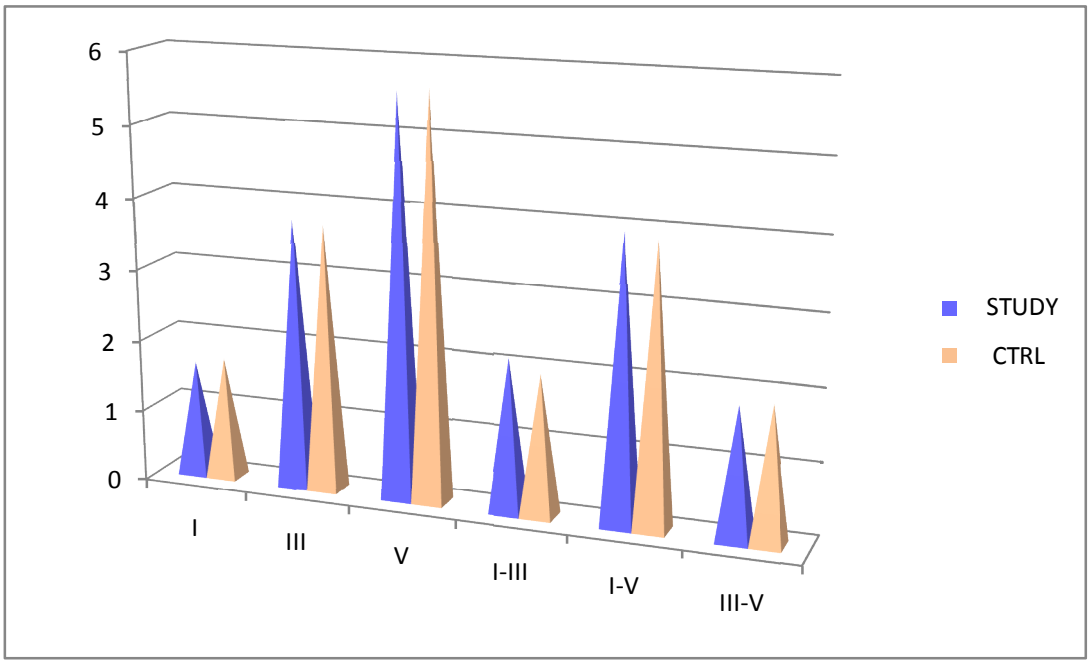
p value – not significant for all parameters.

TABLE 17: Comparison of mean values of interpeak latencies between GTCS males and control males in the left ear

IPL	STUDY GROUP	CONTROL GROUP	T TEST	P VALUE
	MEAN ± SD	MEAN ± SD		
I-III	2.23 ±0.39	2.02 ±0.15	1.94	0.06
I-V	4.06 ±0.26	3.89 ±0.33	1.56	0.12
III-V	1.83 ±0.20	1.89 ±0.36	0.56	0.57

p value – not significant for all parameters.

GRAPH 9: Comparison of mean values of absolute and interpeak latencies between GTCS males and control males in the right ear



GRAPH 10: Comparison of mean values of absolute and interpeak latencies between GTCS males and control males in the left ear

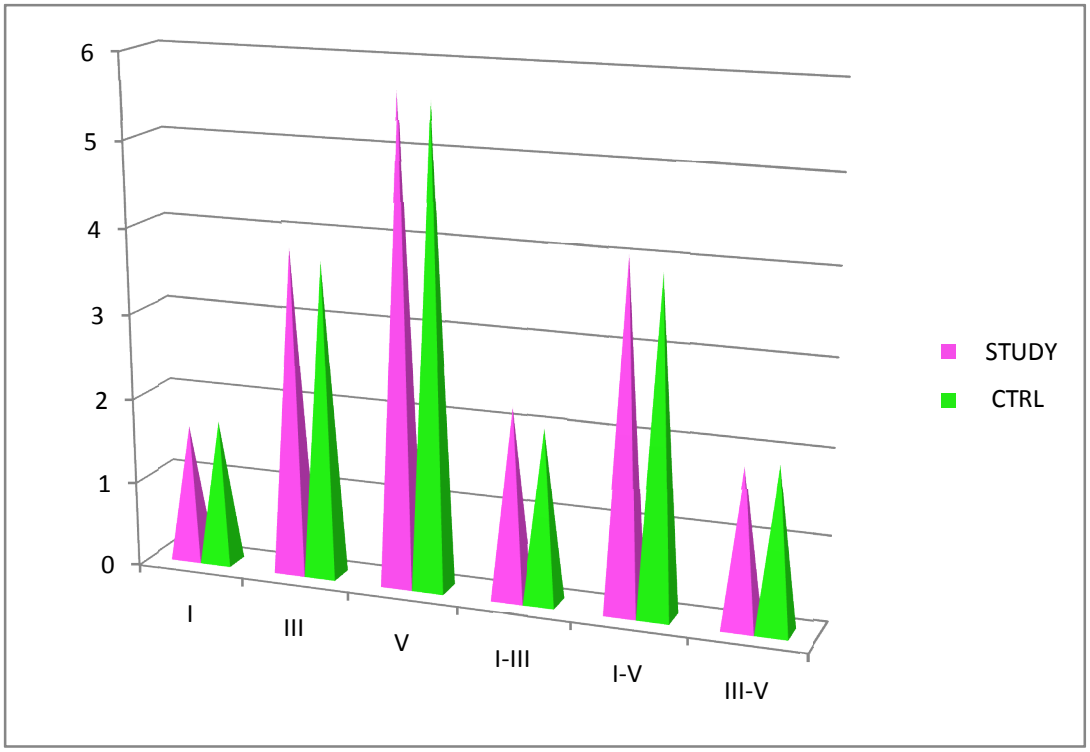


TABLE 18: Comparison of Interleukin-1 beta levels in controls and GTCS patients

GROUPS	**IL-1BETA MEAN±SD in pg**	**P VALUE**
CONTROLS	1.81±0.62	0.039
GTCS PATIENTS	2.15±0.59	

P value< 0.03, significant

The mean level of IL-1beta is significantly higher in GTCS patients when compared with normal controls.

5.4 Correlations between IL-1 beta levels and Brainstem auditory evoked potentials:

The tables 19- 22 show the correlations between IL-1beta levels and brainstem auditory evoked potentials. The correlation coefficients observed were of both negative and weak positive correlations but they were not statistically significant.

GRAPH 11: Comparison of serum inteleukin-1 beta levels in controls and GTCS patients

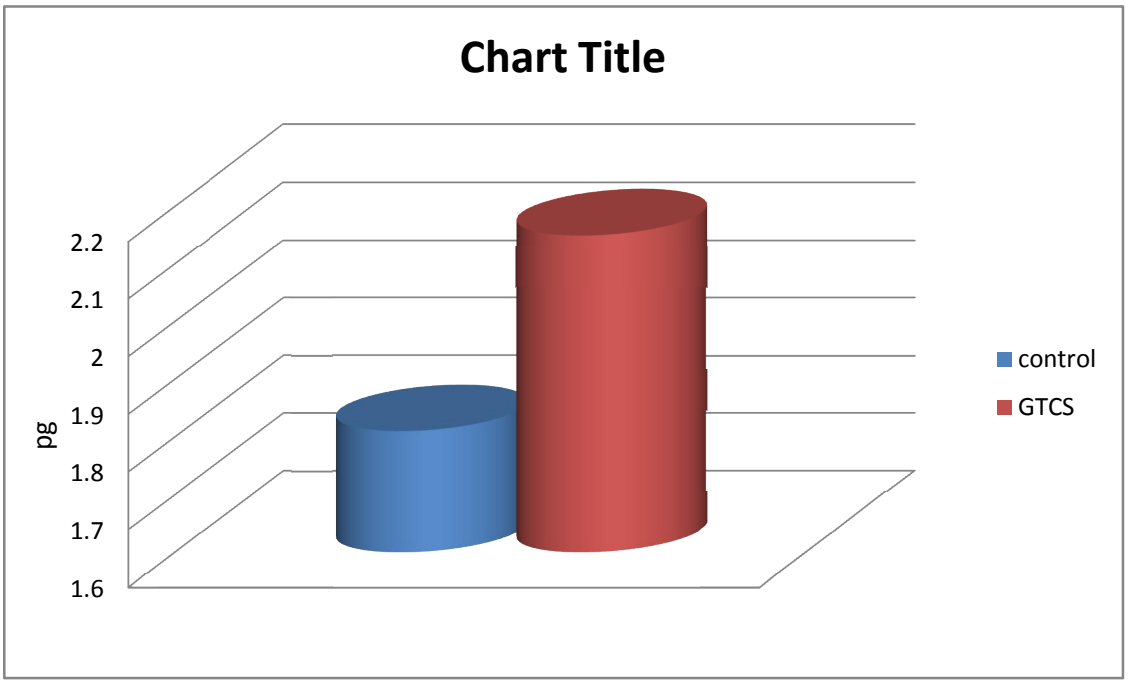


TABLE 19: Correlation between IL-1 beta levels and ABR wave III in the right ear of GTCS patients

	CORRELATION COEFFICIENT	INTERPRETATION
CORRELATION BETWEEN IL-1BETA AND BERA WAVE III (RIGHT EAR)	r = -0.10	Negative correlation
	p = 0.59	Not significant

TABLE 20: Correlation between IL-1 beta levels and ABR wave III in the left ear of GTCS PATIENTS

	CORRELATION COEFFICIENT	INTERPRETATION
CORRELATION BETWEEN IL-1BETA AND BERA WAVE III (LEFT EAR) IN GTCS PATIENTS	r = 0.049	Weak positive correlation
	p = 0.79	Not significant

TABLE 21: Correlation between IL-1 beta levels and ABR I-III IPL in the right ear of GTCS patients

CORRELATION BETWEEN IL-1BETA AND BERA I- III (RIGHT EAR) IN GTCS PATIENTS	CORRELATION COEFFICIENT	INTERPRETATION
	r = -0.18	Negative correlation
	p = 0.32	Not significant

TABLE 22: Correlation between IL-1 beta levels and ABR I-III IPL in the left ear of GTCS patients

CORRELATION BETWEEN IL-1BETA AND BERA I- III (RIGHT EAR) IN GTCS PATIENTS	CORRELATION COEFFICIENT	INTERPRETATION
	r = 0.03	Weak positive correlation
	p = 0.83	Not significant

6.DISCUSSION

6. DISCUSSION

All the GTCS patients and controls included in the study were subjected to Brainstem auditory evoked potential for evaluating the integrity of auditory pathway.

6.1 Characteristics of study subjects

The mean age of GTCS patients included in the study was 30.1±3.11 years. The mean age of GTCS patients in various studies (Usha Panjwani et al 1996, Salah Soliman 1993) are almost similar to that of the present study. Thus the patients in the present study are in the adult group. Beagley et al and Anias et al observed no age related effect on absolute latencies. Therefore it could be stated that any abnormalities of BERA observed in the patients can be attributed to GTCS rather than age related causes.

The duration of disease in GTCS patients of our study ranges from 1-2 years and these patients were on treatment since diagnosed. Soliman, Saad and Haaza[74] observed poor resolution of ABR components and tend to correlate their findings with chronicity of the disease and the possible effects of antiepileptic drugs.

Salah Soliman[98] et al showed that chronicity of epilepsy was positively related to the elevated auditory brainstem response and middle latency response thresholds in grandmal epilepsy patients. Many antiepileptic

drugs produce prolonged absolute latencies and interpeak latencies when administered for a long time. This is in agreement with previous studies of Chan et al[75] and Green et al[108].

The BMI of the GTCS patients was found to be 23.68±1.61. Haslam et al[76] and Landau et al[77] demonstrated that the hypoxic adipose tissue tend to produce inflammatory cytokines; this inflammation results in imbalance between free radical production and its scavenging mechanisms resulting in damage to the neuron and surrounding schwann cells by associated oxidative stress and hence proving obese individuals are prone to develop early damage to auditory nerve.

In the present study, the analysis revealed that there was a significant difference in wave V latency time between the sexes; latencies were higher in the right ear of males when compared to females. This finding is consistent with various published data by Costa Neto et al, Hassan et al and Macedo et al.

Masaru Aoyagi et al[78] proved that head size which reflects brain size is one of the determining factor for the basis of such gender differences.

Taghavy A and Losslein H et al[51] in their study described that absolute latency, interpeak latencies and amplitude of waveforms of BERA were

significantly shorter in females than males. Inter aural differences of the same were negligible.

According to Edmir Americo Louenco et al[126], the absolute latencies of ABR waves were higher in males, whereas the interpeak intervals showed no gender differences and the I-III IPL presented the higher incidence of alterations.

Stockard et al[79] demonstrated experimentally that females have shorter latency which is attributed to higher core body temperature and shorter length of brainstem auditory pathway.

Kellaway et al[80] (1979) observed in women that the auditory evoked potentials show shorter absolute and interpeak latencies and their EEG has a high frequency of the occipital alpha rhythm.

Allison et al[81] (1983) suggested that the length of brainstem auditory pathway varies as the cube root of brain volume and correlating thus with age and sex related variations in brain weight and calculated that the male female ratio should be 1.034+0.0008. When this is compared with normal data, latency sex difference were within normal limits.

Sturzebecher E and Werbs M[127], 1987 found that the latencies of waves of waves I, III & V as well as the interpeak latencies I-V and III-V are significantly shorter in females than in males.

Del Pozo [82](1982) measured the distance between the cochlear nucleus and inferior colliculus in fresh post-mortem specimens; he did find it longer in men, although differences were not statistically significant.

6.2 Brainstem auditory evoked potential

Brainstem auditory evoked potentials reflect the neural activity from the cochlea to temporal cortex and they have been used successfully in clinical practice as an objective means for hearing threshold measurements as well as in the diagnosis of site of lesion in the central auditory nervous system.

Epilepsy is a neurological disorder characterised by abnormal changes in the brain's electrical potentials. In other words, it can be described as cellular level dysfunction leading to excessive neuronal excitability as a result of biophysical and/or biochemical dysfunction. Accordingly auditory evoked potentials are expected to be altered by such dysfunction.

Various studies by Faught et al, Hughes et al, Mervaala et al and Rodin et al have indicated a large variability of AEPs in epileptic patients with various types of epilepsy when compared to normal population.

6.2.1. Wave I

In the present study, the mean absolute latency of wave I is not found to be statistically significant in both right and left ears of male as well as female GTCS patients when compared to controls (1.61±0.15 vs 1.70±0.20 in right ear, 1.60±0.15 vs 1.69±0.19 in left ear, 1.61±0.21 vs 1.64±0.14 in right ear and 1.69±0.12 vs 1.65±0.16 in left ear respectively).

The above finding of our study confirms the absence of any peripheral hearing impairment in the GTCS group which had been clinically ruled out prior to the study by pure tone audiogram. This is consistent with the studies of Ehle et al and Fadly et al as they have suggested that GTCS do not seem to affect the absolute latency of ABR wave I. However controversy to the above finding of our study was shown by Madkow et al in 1998 who had reported shortened wave I absolute latency in epileptic patients.

6.2.2. Wave III

Absolute latency of wave III which reflects the time interval measured in millisec from the onset of stimulus to the peak of wave III, appear to be significantly prolonged in both right and left ears of GTCS patients as compared to controls(3.85±0.23 vs 3.67±0.14 and 3.95±0.20 vs 3.68±0.22 respectively). When analysing the mean values of same latency in both ears of male GTCS patients , it is found to be slightly

prolonged especially in the left ear than controls but not statistically different(3.83±0.27 vs 3.70±0.23). The finding in the present study is similar to the published data of Rodin et al[97] who showed that the GTCS patients had significantly longer wave III absolute latency than the normal controls.

Rodin et al[97] correlated the ABR wave and interpeak latencies with a large variety of clinical variables and showed that statistically significant relationships existed mainly in regard to presence or absence brain damage and the severity of seizure disorder, as expressed by the number of different types of seizures to which a given patient was subject to. They also proved that waves II and III showed the most consistent latency prolongations for these variables.

However controversy to the above finding is suggested by CoS, Levinthal et al[89] and Zakaria et al[99] who identified that epilepsy do not affect the absolute latency of wave III at high intensity. Salem et al[90] also refuted the above finding of the present study by reporting shortened wave III absolute latency and attributed it to the hyperexcitability of the nervous system in epilepsy.

6.2.3. Wave V

On analysing the present study, it is obvious that the absolute latency of ABR wave V which reflects the time interval between stimulus onset and

peak of wave V, is found to be significantly prolonged in both right and left ears of female GTCS patients when compared to controls (5.67±0.17 vs 5.47±0.21, 5.71±0.14 vs 5.46±0.26 respectively). The above finding of the present study is in accordance with Rodin et al[97] who suggested prolonged wave V absolute latency in GTCS patients.

However Usha Panjwani et al[83] and Salem et al[90] contradicted the above findings by demonstrating shortened wave V absolute latency in epileptic group as compared to controls. Also the study by Co S,Leventhal et al[89] elucidates that epilepsy had no significant impact on wave V absolute latency.

6.2.4. IPL I-III

On analysing the data of interpeak latencies in the present study, it is found that that I-III IPL, an index of the conduction time between cochlea and caudal pons is prolonged in both right and left ears of female GTCS patients(2.25±0.31vs 2.03±0.19, 2.26±0.23vs 2.03±0.25 respectively). The mean values of the same IPL is found to be prolonged in both the ears of male GTCS patients but the differences are not statistically significant(2.14±0.35 vs 1.96±0.26, 2.23±0.39 vs 2.02±0.15 respectively).

The above finding is consistent with various studies done by Mervaala etal, Chan et al and Rodin et al. Mervaala et al[100] demonstrated prolonged

I-III IPL and attributed it to the presence of neurological and psychiatric defects in their patient population. Chan et al postulated prolonged ABR I-III IPL and related such delay to the effect of antiepileptic drugs. Rodin et al[97] observed significantly longer I-III IPL in GTCS patients as compared to controls. Auguglia et al[88] refuted this finding of the present study by demonstrating that GTCS do not alter ABR I-III IPL. Also S.H.Singh et al reported reduced I-III IPL but the differences were not statistically different.

6.2.5. IPL I-V

In the present study, the data analysis makes it clear that ABR I-V IPL is significantly prolonged in both right and left ears of female GTCS patients as compared to controls (4.07±0.17vs3.83±0.27, 4.02±0.16 vs 3.80±0.33 respectively). In left ear of male GTCS patients, the same IPL has increased but not statistically significant.

This finding of our study is supported by Rodin et al[97] who demonstrated significantly prolonged I-V IPL in GTCS patients when compared to controls. Also the studies done by Keranen et al, Woo et al and Masayuki Ohishi et al[125] showed findings similar to the present study demonstrating prolonged I-V IPL in GTCS patients.

But in contrast to the present study, Usha Panjwani et al[83] reported shortened I-V IPL as compared to controls. Also Aguglia et al[88] demonstrated normal I-V IPL latency in epilepsy patients.

6.2.6. IPL III-V

On comparing the ABR III-V IPL of both male and female GTCS patients with controls, it is observed that there exists no significant difference between GTCS patients and controls in both ears. This finding makes it obvious to conclude that the I-V IPL prolongation is mainly because of the increase of I-III IPL with III-V IPL(the conduction time from pons to midbrain) remaining as a constant. Rodin et al[97] supports this observation of the present study by suggesting epileptic patients with severe brain damage have demonstrable brainstem dysfunction affecting mainly the medullopontine rather than midbrain or thalamic structures.

There are no statistically significant differences observed when right and left ears of GTCS patients are compared. Fabiano et al also reached similar conclusions. No latency difference between the ears are anticipated in patients with bilaterally normal auditory thresholds.

Engel J et al[84] suggests that epileptogenicity is modulated by mechanisms which can alter the neuronal excitability and synchronisation involving both synaptic and nonsynaptic events. This altered synaptic

transmission and/or neuronal conduction lead to reduced wave V absolute latency and I-V IPL in GTCS patients.

Cosi et al[85] published in his study that epilepsy patients have their BERA values within normal limits.

Usha Panjwani et al[83] emphasised in their study that epilepsy per se may produce alterations in BAEPs. These potentials can be utilised in the evaluation of AED therapy since the test gives comparatively better information about the functional effects of AEDs than blood levels which may not appropriately indicate the effective levels at the CNS.

Comis et al[109], Musiek et al[110] and Watanabe et al[111] observed GABA and Ach as the neurotransmitters of auditory system. Loscher et al[112] and Tower et al[113] proved that imbalance of these neurotransmitters had been correlated with seizure production in epileptic patients. Hence it can be speculated that such imbalance of neurotransmitters could extend to a subcortical level and be responsible for the dysynchrony of the auditory brain stem pathways.

Robinson et al[114] and Selters et al[115] proposed the present criteria for defining auditory brainstem dysfunction which includes prolongation of IPL and /or absence of ABR waves.

Yaari Y et al[86], Durelli L et al and Eggermont JJ et al[87] suggested that a slower conduction velocity which is due to the effects on axon membranes, synaptic transmission and neuronal integration may prolong the central conduction time in GTCS patients. Klutke et al reported prolonged central brainstem conduction time in epileptic patients.

Gestaut et al[116], Kileny et al[117],Scherg et al[118]and Penfield W et al[119] proposed that the thalamocortical pathways and reticular formation are involved in the generation of both MLR auditory evoked potentials and epileptogenic activity. Sharing some generators of ABR waves, the deep epileptogenic foci can directly interfere or inhibit the normal pathway of MLR which is the reason for the diminished accuracy of MLR relative to ABR in threshold estimation of epileptic patients.

6.3 Interleukin-1beta levels

Interleukin -1β is a pro inflammatory cytokine originating peripherally or in the CNS, can modulate neuronal excitability and contribute to epileptogenesis. If the influence of IL-1β on epileptogenesis is established, this cytokine is an important target to explore possible new antiepileptic treatment strategies which are based on interference with intracellular signalling cascade that are initiated when IL-1 β binds to its receptor.

In the present study, the mean serum level of IL-1 β in GTCS patients is 2.15±0.59 pg which is significantly higher when compared to controls indicating its potential role in seizure development. This finding of our study is consistent with the view of S.Sinha et al[91]which strengthen the hypothesis postulating cerebral production of cytokines triggered by seizures.

Lehtimaki et al[101] and Sinha et al[91] observed an increase in serum cytokine levels including IL-I β within a few hours after the seizure episode followed by a trend towards normalisation by 16 days, thus revealing important relationship between seizure and increased cytokine levels. Peltola et al[92] suggested that increased levels of cytokines including IL-1 β are found to be higher after more severe seizure (generalised), suggesting that increased levels of cytokines are causally related to the seizure activity.

But this finding of the present study is refuted by Rijkers et al[93] who attempted to determine IL-1 β in plasma of GTCS patients but concluded no consistent change was noticed between epilepsy patients and controls. Helminen et al[96], Matsuo et al[123] and Straussberg et al[124] proved that blood cells of epilepsy patients do not consistently produce more IL-1β in response to inflammatory stimulation than blood cells of controls.

Bertolani et al[120], Haspolat et al[95] and Virta et al[94] observed no consistent changes in plasma levels of any of the IL-1 family members in

patients with epilepsy. Pacifi et al[121] identified that antiepileptic treatment does not affect IL-1beta production by mononuclear cells along with Hulkkonen et al[122] who acknowledged that antiepileptic drugs do not affect the plasma levels of IL-1β.

6.4 Correlation of variables

There was no significant correlation noted between interleukin-1 beta levels and ABR wave absolute latencies and interpeak latencies. Dunn AJ et al and Fann MJ et al[69] reported cytokines including IL-1β influence many central neurotransmitters including GABA, 5-hydroxy tryptamine, noradrenaline and acetyl choline as well as expression of a number of neuropeptides in several brain regions contributing to changes in auditory evoked potentials of GTCS patients.

The reason for absence of correlation between variables in the present study could be attributed to minimum sample size taken in the study and only a few percentage of GTCS patients fall beyond the mean±3SD value range of normal subjects with respect to all variables assessed in the study.

7. CONCLUSION

7. CONCLUSION

The following conclusions have been derived from the present study.

- It is proved that GTCS causes prolongation of central conduction time since significant variations were found in wave III, V absolute latency and I-III, I-V IPL when compared to normal individuals.
- It is characterised by demonstrable brainstem dysfunction affecting mainly the medullopontine rather than thalamic or midbrain structures.
- Epileptogenicity is the outcome of neurotransmitter imbalance which extends to the subcortical level and be responsible for the dyssynchrony of the auditory brainstem pathway.
- The cytokine levels increases significantly in GTCS patients suggesting the ccurrence of inflammation in seizures.
- Since IL-1β activated pathways contribute to epileptogenesis, the antagonists can be targeted as novel anticonvulsant drugs.
- Elevated IL-1β levels noted in GTCS patients could be the probable cause of the observed abnormalities in BERA.
- Brainstem evoked response audiometry has emerged as a notable tool of investigation in studying the central conduction time of epileptic

patients and hence may be used to study the functional effects of anti epileptic drugs.

Using BAEP, we can easily detect the early changes occurring in the auditory pathway even before the clinical manifestation of hearing deficit occurs so that proper measures to intervene the disease at the earliest possible is achieved which brings down the morbidity and provide a better quality of life for GTCS patients. So BAEP should be recommended as a routine diagnostic procedure in GTCS patients.

8.SUMMARY

8. SUMMARY

Brainstem evoked response audiometry is a simple, effective and noninvasive means of evaluating the functional status of the auditory nerve and brainstem auditory sensory pathway. The present study was conducted in the Institute of Physiology and Experimental medicine, Madras Medical College, Chennai to assess the functional integrity of auditory pathway by recording auditory evoked potentials in Generalised tonic clonic seizure patients and to elucidate the role of Interleukin-1β in the pathogenesis of seizures, in comparison with normal individuals.

30 GTCS patients and 30 normal subjects participated voluntarily in the study and they were subjected to BERA, thereby recording Brain auditory evoked potentials. The serum level of interleukin-1β was also measured. Statistically significant differences in wave III, V absolute latency and I-III, I-V IPL were observed in GTCS patients indicating prolonged central conduction time in spite of these patients having normal hearing sensitivity assessed by pure-tone audiogram prior to the study.

The IL-1β levels were significantly higher in GTCS patients when compared to controls suggesting the proconvulsant action of this inflammatory cytokine and this could be the probable cause of BAEP abnormalities noted in these patients.

Even though BAEP is in clinical use since 1970s and has got a substantial literature to support its efficacy, there is still a paucity of data with regard to this investigational tool in patients with seizures. A few published data in this field of research also show variable results. From the observations of present study, it is clear that GTCS has a significant influence on BAEPs. So BAEP may be utilised as a routine objective tool in characterising the electrophysiological phenomena of neural excitation, conduction and transmission across the auditory pathway.

However, the study has got its own limitations as the above findings need to be confirmed with a larger sample size. Further research is required to reveal the mechanism of nerve injury in GTCS patients and whether this occurs in all types of seizures other than GTCS.

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.G.Savitha,
Postgraduate
Institute of Physiology and Experimental Medicine,
Madras Medical College, Chennai-3.

Dear **Dr.G.Savitha,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Evaluation of Brainstem auditory evoked potential and serum Interleukin-1 Beta levels in patients with Generalised Tonic Clonic Seizures"** No.06042014.

The following members of Ethics Committee were present in the meeting held on 11.03.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr. C.Rajendran, M.D,	-- Chairperson
2.	Prof. Kalaiselvi, M.D, Vice Principal, MMC, Ch-3	-- Member Secretary
3.	Prof. Nandhini, M.D, Inst. of Pharmacology, MMC, Ch-3	-- Member
4.	Prof.Bhavani Sankar, M.S, Prof & HOD General Surgery, MMC, Ch-3	-- Member
5.	Prof.V.Padmavathi, M.D, I/c. Director of Pathology, MMC, Ch-3	-- Member
6.	Thiru. S. Govindasamy, BA., BL	-- Lawyer
7.	Tmt.Arnold Saulina, MA MSW	-- Social Scientist
8.	Thiru.S.Ramesh Kumar, Administrative Officer, MMC, Ch-3.	-- Lay Person

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

21/4/14

Member Secretary, Ethics Committee

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

INFORMED CONSENT FORM

Title of the study: "Evaluation of Brainstem auditory evoked potential and serum interleukin 1 beta levels in patients with generalised tonic clonic seizures"

Name of the Participant:

Name of the Principal Investigator: Dr.G.Savitha

Name of the Institution:

Institute of Physiology and Experimental Medicine,
Madras Medical College and Govt. General Hospital,
Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"Evaluation of Brainstem auditory evoked potential and serum Interleukin 1 beta levels in patients with generalised tonic clonic seizures"

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.

6. I have been advised about the risks associated with my participation in this study.

7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.

8. I have not participated in any research study within the past _____month(s).

9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

13. I have understood that my identity will be kept confidential if my data are publicly presented.

14. I have had my questions answered to my satisfaction.

15. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

INFORMATION TO PARTICIPANTS

Investigator: Dr.G.Savitha

Name of Participant:

Title: "Evaluation of Brainstem auditory evoked potential and serum interleukin 1 beta levels in patients with generalised tonic clonic seizures"

You are invited to take part in this research/ study /procedures. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

You are being asked to participate in this study being conducted in

Institute of Physiology and Experimental Medicine,

Madras Medical College and Govt. General Hospital,

Chennai - 3

What is the Purpose of the Research?

Generalised tonic clonic seizures is a chronic neurological disorder characterised by tonic and clonic phases frequently accompanied by the presence of hearing deficit. Epilepsy is associated with suppressive effects on auditory pathway and hence we want to assess the brainstem auditory evoked potential in patients with generalised tonic clonic seizures and correlate the serum interleukin 1 beta levels with brainstem auditory evoked potential in generalised tonic clonic seizures so that any hearing deficit if diagnosed can be intervened as early as possible.

The Study Design

30 patients with generalised tonic clonic seizures will be selected for the study.

Study Procedures

The study involves assessment of Brainstem auditory evoked potential and serum interleukin 1beta levels.

You will be required to visit the hospital once during the study.

5ml of blood will be collected simultaneously during the study. Blood collection involves prick with a needle and syringe.

In addition, if you notice any physical or mental changes, you must contact the persons listed at the end of the document.

You may have to come to the hospital (study site) for examination and investigations apart from your scheduled visits, if required.

Possible Risks to you - Nil

Possible benefits to you- Hearing deficit can be diagnosed at an early stage so that proper intervention can be taken.

Possible benefits to other people

The result of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefits to future patients.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, IEC and any person or agency required by law like the Drug Controller General of India to view your data, if required.

The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

CONCLUSION

There was significant prolongation of central conduction time in GTCS patients even though there was no clinical evidence of hearing impairment assessed by pure tone audiogram prior to the study. Hence BAEP can be utilized as an objective electrophysiological tool to evaluate the functional integrity of auditory pathway from the external ear to lower brainstem.

KEY WORDS

Generalized tonic clonic seizures, Brainstem auditory evoked potential, Absolute latency, Interpeak latency.

How will your decision to not participate in the study affect you?

Your decisions to not to participate in this research study will not affect your medical care or your relationship with investigator or the institution. Your doctor will still take care of you and you will not lose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start?

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during course of the study without giving any reasons.

However, it is advisable that you talk to the research team prior to stopping the treatment

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

ANNEXURES

PROFORMA

1. Name :
2. Age:
3. Sex:
4. Address :
5. Occupation :
6. Complaints/duration:
7. History of present illness:
8. History of any hearing problem after the onset of seizures?
9. Past history:
10. History of any drug intake
11. History of associated illness:
    a. Diabetes
    b. Hypertension
    c. Ischemic heart disease
    d. Respiratory diseases
    e. Renal diseases

Investigations:

1. Fasting Blood sugar
2. Serum electrolytes
3. Pure tone audiometry

General examination:

Temperature:

Pulse rate:

Blood pressure:

Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:

ENT examination:

Brainstem auditory evoked potential study:

Date of conduct of study:

MASTER CHART

MASTER CHART FOR GTCS PATIENTS

SNO	AGE	SEX	BMI	LATENCY IN ms (RIGHT EAR)						LATENCY IN ms (LEFT EAR)						IL-1β
				Wave I	Wave III	Wave V	IPL I-III	IPL I-V	IPL III-V	Wave I	Wave III	Wave V	IPL I-III	IPLI-V	IPL III-V	Pg/ml
1	33	M	23	1.7	3.99	5.6	2.29	3.9	1.61	1.31	3.88	5.69	2.57	4.38	1.81	2.22
2	34	M	23.4	1.6	3.41	5.4	1.81	3.8	1.99	1.48	3.93	5.72	2.45	4.24	1.79	2.93
3	32	M	25.5	1.4	3.94	5.58	2.54	4.18	1.64	1.56	4.08	5.86	2.52	4.3	1.78	1.43
4	28	M	25	1.5	3.22	5.7	1.72	4.2	2.48	1.49	3.99	5.81	2.5	4.32	1.82	2.42
5	33	M	27	1.5	3.96	5.67	2.46	4.17	1.71	1.77	3.41	5.49	1.64	3.72	2.08	1.45
6	37	M	23.5	1.6	3.95	5.64	2.35	4.04	1.69	1.69	3.44	5.61	1.75	3.92	2.17	2.11
7	35	M	23.5	1.7	3.61	5.53	1.91	3.83	1.92	1.8	3.32	5.63	1.52	3.83	2.31	1.46
8	29	M	22	1.9	3.71	5.52	1.81	3.62	1.81	1.63	4.15	5.76	2.52	4.13	1.61	2.92
9	29	M	27.5	1.6	4.07	5.78	2.47	4.18	1.71	1.73	4.13	5.8	2.4	4.07	1.67	2.13
10	33	M	22	1.8	3.09	5.01	1.29	3.21	1.92	1.81	3.57	5.26	1.76	3.45	1.69	2.95
11	34	M	25	1.4	3.81	5.59	2.41	4.19	1.78	1.4	3.99	5.71	2.59	4.31	1.72	2.95
12	35	M	24.7	1.6	3.99	5.78	2.39	4.18	1.79	1.48	3.98	5.71	2.5	4.23	1.73	1.91
13	31	M	25.9	1.4	3.71	5.52	2.31	4.12	1.81	1.65	4.02	5.7	2.37	4.05	1.68	1.23
14	29	M	26.9	1.8	3.95	5.76	2.15	3.96	1.81	1.48	4.01	5.66	2.53	4.18	1.65	1.21
15	33	M	24.9	1.7	4.01	5.79	2.31	4.09	1.78	1.77	3.67	5.64	1.9	3.87	1.97	2.91
16	34	F	22.2	1.85	3.77	5.77	1.92	3.92	2	1.77	3.52	5.88	1.75	4.11	2.36	2.04
17	35	F	24.5	1.9	3.52	5.94	1.62	4.04	2.42	1.47	3.88	5.4	2.41	3.93	1.52	2.95
18	29	F	23.3	1.42	4	5.79	2.58	4.37	1.79	1.92	4.08	5.69	2.16	3.77	1.61	2.81
19	28	F	24.3	1.69	3.81	5.59	2.12	3.9	1.78	1.78	4.21	5.84	2.43	4.06	1.63	2.15
20	21	F	25.6	1.73	3.6	5.74	1.87	4.01	2.14	1.62	4.1	5.8	2.48	4.18	1.7	1.3
21	28	F	24.5	1.5	3.64	5.84	2.14	4.34	2.2	1.51	3.92	5.53	2.41	4.02	1.61	2.91
22	35	F	25	1.92	4.03	5.75	2.11	3.83	1.72	1.65	4.02	5.65	2.37	4	1.63	2.78
23	33	F	23.9	1.42	3.52	5.43	2.1	4.01	1.91	1.67	4.08	5.69	2.41	4.02	1.61	1.67
24	33	F	23.4	1.9	4.21	5.84	2.31	3.94	1.63	1.73	3.61	5.83	1.88	4.1	2.22	2.01
25	38	F	22.6	1.56	4.23	5.89	2.67	4.33	1.66	1.88	4.02	5.6	2.14	3.72	1.58	2.07
26	24	F	22.4	1.42	3.71	5.42	2.29	4	1.71	1.61	4.15	5.86	2.54	4.25	1.71	2.34
27	28	F	23.1	1.6	4.05	5.69	2.45	4.09	1.64	1.65	3.71	5.91	2.06	4.26	2.2	2.23
28	25	F	26.6	1.42	3.82	5.43	2.4	4.01	1.61	1.79	3.95	5.57	2.16	3.78	1.62	1.34
29	34	F	22.3	1.33	3.95	5.47	2.62	4.14	1.52	1.77	4.11	5.79	2.34	4.02	1.68	1.41
30	28	F	23.4	1.4	4.02	5.65	2.62	4.25	1.63	1.58	4.01	5.7	2.43	4.12	1.69	2.46

MASTER CHART FOR CONTROLS

SNO	AGE	SEX	BMI	LATENCY IN ms (RIGHT EAR)						LATENCY IN ms (LEFT EAR)						IL-1β
				Wave I	Wave III	Wave V	IPL I-III	IPL I-V	IPL III-V	Wave I	Wave III	Wave V	IPL I-III	IPL I-V	IPL III-V	Pg/ml
1	35	M	23.1	1.77	3.94	5.7	2.2	3.92	1.75	1.42	3.4	5.6	1.9	4.18	2.2	1.35
2	29	M	24.2	1.54	3.79	6	2	3.92	1.79	1.65	3.7	6.1	2.05	4.5	2.4	2.7
3	37	M	25.3	1.65	3.73	5.6	2.1	3.92	1.83	1.73	3.5	5.4	1.8	3.6	1.9	1.35
4	36	M	26.6	1.63	3.29	5.4	1.66	3.77	2.11	1.67	3.3	5.6	2.03	3.93	2.3	2.5
5	39	M	23.3	1.69	3.69	5.5	2	3.81	1.81	1.42	3.9	5.4	2.5	3.9	1.46	1.31
6	38	M	26	1.92	3.9	6	2	4.1	2.13	1.77	3.9	5.7	2.13	3.93	1.8	1.21
7	24	M	24.1	1.65	3.85	5.8	2.2	4.17	1.96	1.42	3.5	5.4	2.08	3.98	1.9	1.31
8	32	M	23.5	1.9	3.69	5.6	1.8	3.67	1.88	1.56	3.6	5.7	2.04	4.14	2.1	1.55
9	31	M	24.9	1.42	3.69	5.4	1.8	3.98	1.71	1.69	3.7	5.4	2.01	3.71	1.7	1.89
10	36	M	24.5	1.98	3.81	5.6	1.83	3.62	1.79	1.65	3.6	6.15	1.95	4.5	2.55	1.92
11	26	M	22.9	1.77	3.73	5.8	2	4	2.04	1.77	3.7	5.6	1.93	3.83	1.9	2.9
12	28	M	24.5	1.31	3.98	5.7	2.7	4.42	1.75	1.9	3.9	5.3	2	3.4	1.4	1.9
13	34	M	24.7	1.98	3.75	5.5	1.77	3.52	1.75	1.81	3.9	5.3	2.09	3.5	1.4	1.72
14	36	M	24.5	1.52	3.44	5.4	1.92	3.88	1.96	2.17	4.2	5.73	2.03	3.56	1.53	1.61
15	29	M	26	1.85	3.51	5.6	1.66	3.75	2.09	1.77	3.7	5.6	1.9	3.83	1.9	2.11
16	33	F	23.4	1.42	3.44	5.8	2.02	4.38	2.36	1.65	3.7	5.52	2.1	3.87	1.82	1.1
17	31	F	22.9	1.69	3.9	5.6	2.2	3.91	1.7	1.77	3.5	5.6	1.7	3.83	2.1	1.91
18	28	F	24.5	1.77	3.56	5.3	1.8	3.53	1.74	1.94	3.7	5.21	1.8	3.27	1.51	1.87
19	21	F	25.4	1.73	3.48	5.2	1.75	3.47	1.72	1.6	3.6	5.31	2	3.71	1.71	1.78
20	22	F	24.2	1.6	3.73	5.6	2.1	4	1.87	1.77	3.4	5.35	1.6	3.58	1.95	2.31
21	24	F	25.1	1.73	3.6	5.4	1.9	3.67	1.8	1.44	3.7	6.15	2.3	4.71	2.45	1.56
22	27	F	24.1	1.63	3.94	5.2	2.3	3.57	1.26	1.56	3.4	5.23	1.8	3.67	1.83	2.8
23	33	F	23.4	1.85	3.73	5.7	1.9	3.85	1.97	1.73	4.2	5.65	2.5	3.92	1.45	1.56
24	32	F	23.7	1.65	3.6	5.4	2	3.75	1.8	1.33	3.4	5.19	2.1	3.86	1.79	1.21
25	37	F	23.9	1.63	3.77	5.4	2.1	3.77	1.63	1.54	3.9	5.4	2.4	3.86	1.5	1.87
26	35	F	25.5	1.48	3.67	5.1	2.2	3.62	1.43	1.6	3.7	5.6	2.1	4	1.9	1.76
27	34	F	23.1	1.77	3.73	5.7	2	3.93	1.97	1.94	3.9	5.25	1.9	3.31	1.35	1.61
28	26	F	24.1	1.42	3.6	5.6	2.2	4.18	2	1.67	3.6	5.69	1.9	4.02	2.09	2.87
29	29	F	23.1	1.48	3.77	5.7	2.3	4.22	1.93	1.56	3.7	5.19	2.1	3.63	1.49	1.72
30	24	F	23.4	1.81	3.5	5.4	1.7	3.59	1.9	1.73	3.9	5.6	2.2	3.87	1.7	1.13

KEY TO MASTER CHART

BMI	-	Body Mass Index
IPL	-	Interpeak Latency
IL-1β	-	Interleukin – 1 Beta
ms	-	Milliseconds